PCT

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :		(11) International Publication Number:	**WO 97/26853**
A61K	**A2**	(43) International Publication Date:	31 July 1997 (31.07.97)

(21) International Application Number: PCT/US97/01301

(22) International Filing Date: 24 January 1997 (24.01.97)

(30) Priority Data:
08/590,494	24 January 1996 (24.01.96)	US
08/626,046	1 April 1996 (01.04.96)	US
08/626,685	1 April 1996 (01.04.96)	US
08/721,837	27 September 1996 (27.09.96)	US

(60) Parent Applications or Grants
(63) Related by Continuation
US	08/721,837 (CIP)
Filed on	27 September 1996 (27.09.96)
US	08/626,685 (CIP)
Filed on	1 April 1996 (01.04.96)
US	08/626,046 (CIP)
Filed on	1 April 1996 (01.04.96)
US	08/590,494 (CIP)
Filed on	24 January 1996 (24.01.96)

(71) Applicant (for all designated States except US): SYNAPTIC PHARMACEUTICAL CORPORATION [US/US]; 215 College Road, Paramus, NJ 07652 (US).

(72) Inventors; and
(75) Inventors/Applicants (for US only): SMITH, Kelli, E. [US/US]; 401 Riverside Drive, Wayne, NJ 07470 (US). GERALD, Christophe, P., G. [FR/US]; 204B Union Street, Ridgewood, NJ 07450 (US). WEINSHANK, Richard, L. [US/US]; 268 Vandelinda Avenue, Teaneck, NJ 07666 (US). LINEMEYER, David [US/US]; 105 Melba Street, Milford, CT 06460 (US). BRANCHEK, Theresa [US/US]; 518 Standish Road, Teaneck, NJ 07666 (US). FORRAY, Carlos [CL/US]; 304 Spring Valley Road, Paramus, NJ 07652 (US).

(74) Agent: WHITE, John, P.; Cooper & Dunham, L.L.P., 1185 Avenue of the Americas, New York, NY 10036 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

(54) Title: DNA ENCODING GALANIN GALR2 RECEPTORS AND USES THEREOF

(57) Abstract

This invention provides isolated nucleic acids encoding mammalian galanin receptors, isolated galanin receptor proteins, vectors comprising isolated nucleic acid encoding a mammalian galanin receptor, cells comprising such vectors, antibodies directed to a mammalian galanin receptor, nucleic acid probes useful for detecting nucleic acid encoding a mammalian galanin receptor, antisense oligonucleotides complementary to unique sequences of nucleic acid encoding a mammalian galanin receptor, nonhuman transgenic animals which express DNA encoding a normal or a mutant mammalian galanin receptor, as well as methods of determining binding of compounds to mammalian galanin receptors.

DNA ENCODING GALANIN GALR2 RECEPTORS AND USES THEREOF

5   This application is a continuation-in-part in the U.S. of
U.S. Serial No. 08/721,837, filed September 27, 1996,
which is a continuation-in-part of U.S. Serial No.
08/626,685 and U.S. Serial No. 08/626,046, both filed
April 1, 1996, which are continuations-in-part of U.S.
10   Serial No. 08/590,494, filed January 24, 1996, the
contents of which are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

Throughout this application, various references are
15   referred to within parentheses. Disclosures of these
publications in their entireties are hereby incorporated
by reference into this application to more fully describe
the state of the art to which this invention pertains.
Full bibliographic citation for these references may be
20   found at the end of this application, preceding the
sequence listing and the claims.

The neuropeptide galanin and its receptors hold great
promise as targets for the development of novel
25   therapeutic agents. Galanin is widely distributed
throughout the peripheral and central nervous systems and
is associated with the regulation of processes such as
somatosensory transmission, smooth muscle contractility,
hormone release, and feeding (for review, see Bartfai et
30   al., 1993). In the periphery galanin is found in the
adrenal medulla, uterus, gastrointestinal tract, dorsal
root ganglia (DRG), and sympathetic neurons. Galanin
released from sympathetic nerve terminals in the pancreas
is a potent regulator of insulin release in several
35   species (Ahrén and Lindskog, 1992; Boyle et al., 1994),
suggesting a potential role for galanin in the etiology
or treatment of diabetes. High levels of galanin are
observed in human and rat anterior pituitary where
galanin mRNA levels are potently upregulated by estrogen
40   (Vrontakis et al., 1987; Kaplan et al., 1988). The

- 2 -

presence of galanin in the hypothalamic-pituitary-adrenal
axis coupled with its potent hormonal effects has led to
the suggestion that galanin may play an integral role in
the hormonal response to stress (Bartfai et al., 1993).

5

Within the CNS galanin-containing cell bodies are found
in the hypothalamus, hippocampus, amygdala, basal
forebrain, brainstem nuclei, and spinal cord, with
highest concentrations of galanin in the hypothalamus and
10   pituitary (Skofitsch and Jacobowitz, 1985; Bennet et al.,
1991; Merchenthaler et al., 1993). The distribution of
galanin receptors in the CNS generally complements that
of galanin peptide, with high levels of galanin binding
observed in the hypothalamus, amygdala, hippocampus,
15   brainstem and dorsal spinal cord (Skofitsch et al., 1986;
Merchenthaler et al., 1993; see Bartfai et al., 1993).
Accordingly, agents modulating the activity of galanin
receptors would have multiple potential therapeutic
applications in the CNS. One of the most important of
20   these is the regulation of food intake. Galanin injected
into the paraventricular nucleus (PVN) of the
hypothalamus stimulates feeding in satiated rats
(Kyrkouli et al., 1990), an effect which is blocked by
the peptide galanin antagonist M40 (Crawley et al.,
25   1993). In freely feeding rats, PVN injection of galanin
preferentially stimulates fat-preferring feeding (Tempel
et al., 1988); importantly, the galanin antagonist M40
administered alone decreases overall fat intake
(Leibowitz and Kim, 1992). These data indicate that
30   specific receptors in the hypothalamus mediate the
effects of galanin on feeding behavior, and further
suggest that agents acting at hypothalamic galanin
receptors may be therapeutically useful in the treatment
of human eating disorders.

35

Galanin receptors elsewhere in the CNS may also serve as
therapeutic targets. In the spinal cord galanin is

released from the terminals of sensory neurons as well as spinal interneurons and appears to play a role in the regulation of pain threshold (Wiesenfeld-Hallin et al., 1992). Intrathecal galanin potentiates the anti-
5   nociceptive effects of morphine in rats and produces analgesia when administered alone (Wiesenfeld-Hallin et al., 1993; Post et al., 1988); galanin receptor agonists may therefore be useful as analgesic agents in the spinal cord. Galanin may also play a role in the development of
10   Alzheimer's disease. In the hippocampus galanin inhibits both the release (Fisone et al., 1987) and efficacy (Palazzi et al., 1988) of acetylcholine, causing an impairment of cognitive functions (Sundström et al., 1988). Autopsy samples from humans afflicted with
15   Alzheimer's disease reveal a galaninergic hyperinnervation of the nucleus basalis (Chan-Palay, 1988), suggesting a role for galanin in the impaired cognition characterizing Alzheimer's disease. Together these data suggest that a galanin antagonist may be
20   effective in ameliorating the symptoms of Alzheimer's disease (see Crawley, 1993). This hypothesis is supported by the report that intraventricular administration of the peptide galanin antagonist M35 improves cognitive performance in rats (Ögren et al.,
25   1992). Human galanin receptors thus provide targets for therapeutic intervention in multiple CNS disorders.

High-affinity galanin binding sites have been characterized in brain, spinal cord, pancreatic islets
30   and cell lines, and gastrointestinal smooth muscle in several mammalian species, and all show similar affinity for ^{125}I-porcine galanin (~0.5-1 nM). Nevertheless, recent in vitro and in vivo pharmacological studies in which fragments and analogues of galanin were used suggest the
35   existence of multiple galanin receptor subtypes. For example, a galanin binding site in guinea pig stomach has been reported that exhibits high affinity for porcine

- 4 -

galanin (3-29) (Gu, et al. 1995), which is inactive at
CNS galanin receptors. The chimeric galanin analogue M15
(galantide) acts as antagonist at CNS galanin receptors
(Bartfai et al., 1991) but as a full agonist in
gastrointestinal smooth muscle (Gu et al., 1993).
Similarly, the galanin-receptor ligand M40 acts as a weak
agonist in RINm5F insulinoma cells and a full antagonist
in brain (Bartfai et al, 1993a). The pharmacological
profile of galanin receptors in RINm5F cells can be
further distinguished from those in brain by the
differential affinities of [D-Tyr^2]- and [D-Phe^2]-galanin
analogues (Lagny-Pourmir et al., 1989). The chimeric
galanin analogue M35 displaces ^{125}I-galanin binding to
RINm5F membranes in a biphasic manner, suggesting the
presence of multiple galanin receptor subtypes, in this
cell line (Gregersen et al., 1993).

Multiple galanin receptor subtypes may also co-exist
within the CNS. Galanin receptors in the dorsal
hippocampus exhibit high affinity for Gal (1-15) but not
for Gal (1-29) (Hedlund et al., 1992), suggesting that
endogenous proteolytic processing may release bioactive
fragments of galanin to act at distinct receptors. The
rat pituitary exhibits high-affinity binding for ^{125}I-
Bolton and Hunter (N-terminus)-labeled galanin (1-29) but
not for [^{125}I]Tyr^{26}-porcine galanin (Wynick et al., 1993),
suggesting that the pituitary galanin receptor is a C-
terminus-preferring subtype. Spinal cord galanin binding
sites, while similar to those in brain, show an affinity
for the chimeric peptide antagonist M35 intermediate
between the brain and smooth muscle (Bartfai et al.,
1991), raising the possibility of further heterogeneity.

A galanin receptor cDNA was recently isolated by
expression cloning from a human Bowes melanoma cell line
(Habert-Ortoli et al., 1994). The pharmacological
profile exhibited by this receptor is similar to that

- 5 -

observed in brain and pancreas, and on that basis the receptor has been termed GALR1. The cloned human GALR1 receptor binds native human, porcine and rat galanin with ~1 nM affinity (K_i vs. ^{125}I-galanin) and porcine galanin 1-

5   16 at a slightly lower affinity (~5nM). Porcine galanin 3-29 does not bind to the receptor. The GALR1 receptor appears to couple to inhibition of adenylate cyclase, with half-maximal inhibition of forskolin-stimulated cAMP production by 1 nM galanin, and maximal inhibition

10   occurring at about 1 μM.

Recently the rat homologue of GALR1 was cloned from the RIN14B pancreatic cell line (Burgevin, et al., 1995, Parker et al., 1995; Smith et al., in preparation). The

15   pharmacological data reported to date do not suggest substantial differences between the pharmacologic properties of the rat and human GALR1 receptors. Localization studies reveal GALR1 mRNA in rat hypothalamus, ventral hippocampus, brainstem, and spinal

20   cord (Gustafson et al., 1996), regions consistent with roles for galanin in feeding, cognition, and pain transmission. However, GALR1 appears to be distinct from the pituitary and hippocampal receptor subtypes described above.

25

The indication of multiple galanin receptor subtypes within the brain underscores the importance of defining galanin receptor heterogeneity at the molecular level in order to develop specific therapeutic agents for CNS

30   disorders. Pharmacological tools capable of distinguishing galanin receptor subtypes in tissue preparations are only beginning to appear. Several high-affinity peptide-based galanin antagonists have been developed and are proving useful in probing the functions

35   of galanin receptors (see Bartfai et al., 1993), but their peptide character precludes practical use as therapeutic agents. In light of galanin's multiple

- 6 -

neuroendocrine roles, therapeutic agents targeting a
specific disorder must be selective for the appropriate
receptor subtype to minimize side effects.

5   Accordingly, the cloning of the entire family of galanin
receptors for use in target-based drug design programs
has been endeavored. The identification of non-peptide
agents acting selectively only at specific galanin
receptors will be greatly facilitated by the cloning,
10   expression, and characterization of the galanin receptor
family.

The isolation by expression cloning of a novel galanin
receptor from a rat hypothalamic cDNA library, as well as
15   its pharmacological characterization in a heterologous
expression system is now reported. The data provided
demonstrate for the first time the existence of a new
galanin receptor subtype, from now on referred to as the
GALR2 subtype, or simply, "GALR2." The cloning of the
20   human homolog of the rat GALR2 receptor is also reported.
This discovery provides a novel approach, through the use
of heterologous expression systems, to develop subtype
selective, high-affinity non-peptide compounds that could
serve as therapeutic agents for eating disorders,
25   diabetes, pain, depression, ischemia, and Alzheimer's
disease. The presence of both GALR1 and GALR2 in rat
hypothalamus suggests that multiple galanin receptors may
be involved in the regulation of feeding.
Pathophysiological disorders proposed to be linked to
30   galanin receptor activation include eating disorders,
diabetes, pain, depression, ischemia, Alzheimer's disease
and reproductive disorders. Accordingly, treatment of
such disorders may be effected by the administration of
GALR2 receptor-selective compounds. The localization of
35   GALR2 receptors in other parts of the rat brain suggests
that GALR2 receptors may play a role in cognition,
analgesia, sensory processing (olfactory, visual),

processing of visceral information, motor coordination, modulation of dopaminergic activity, neuroendocrine function, sleep disorders, migraine, and anxiety.

- 8 -

SUMMARY OF THE INVENTION

This invention provides an isolated nucleic acid encoding
a mammalian GALR2 galanin receptor. This invention also
provides an isolated GALR2 receptor protein. This
invention further provides DNA, cDNA, genomic DNA, RNA,
and mRNA encoding the GALR2 receptor.

This invention further provides a vector comprising the
GALR2 receptor. This invention also provides a plasmid
which comprises the regulatory elements necessary for
expression of GALR2 nucleic acid in a mammalian cell
operatively linked to a nucleic acid encoding the GALR2
receptor so as to permit expression thereof, designated
K985 (ATCC Accession No. 97426). This invention also
provides a plasmid which comprises the regulatory
elements necessary for expression of GALR2 nucleic acid
in a mammalian cell operatively linked to a nucleic acid
encoding the GALR2 receptor so as to permit expression
thereof, designated BO29 (ATCC Accession No. 97735). This
invention provides mammalian cells comprising the above-
described plasmid or vector. This invention also
provides a membrane preparation isolated from the cells.

This invention provides a nucleic acid probe comprising
at least 15 nucleotides, which probe specifically
hybridizes with a nucleic acid encoding a GALR2 receptor,
wherein the probe has a unique sequence corrresponsing to
a sequence present within one of the two strands of the
nucleic acid encoding the GALR2 receptor contained in
plasmid K985, plasmid BO29, plasmid BO39 or plasmid
K1045. In one embodiment, the GALR2 receptor is the rat
GALR2 receptor encoded by the coding sequence of plasmid
K985. In another embodiment, the GALR2 receptor is the
human GALR2 receptor encoded by the coding sequence of
plasmid BO29. This invention also provides a nucleic
acid probe comprising at least 15 nucleotides, which
probe specifically hybridizes with a nucleic acid

- 9 -

encoding a GALR2 receptor, wherein the probe has a unique sequence corresponding to a sequence within (a) the nucleic acid sequence shown in Figure 1 or Figure 10, or (b) the reverse complement of the nucleic acid sequence shown in Figure 1 or Figure 10. This invention further provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a unique fragment of the sequence of a nucleic acid molecule encoding a GALR2 receptor. This invention also provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to the antisense sequence of a unique fragment of the sequence of a nucleic acid molecule encoding a GALR2 receptor.

This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to mRNA encoding a GALR2 galanin receptor, so as to prevent translation of the mRNA. This invention also provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA molecule encoding a GALR2 receptor.

This invention provides an antibody directed to a GALR2 receptor. This invention also provides a monoclonal antibody directed to an epitope of a GALR2 receptor, which epitope is present on the surface of a cell expressing a GALR2 receptor.

This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide effective to reduce activity of a GALR2 receptor by passing through a cell membrane and binding specifically with mRNA encoding a GALR2 receptor in the cell so as to prevent its translation and a pharmaceutically acceptable carrier capable of passing through a cell membrane. In an embodiment, the oligonucleotide is coupled to a substance

- 10 -

which inactivates mRNA. In another embodiment, the substance which inactivates mRNA is a ribozyme.

5   This invention provides a pharmaceutical composition comprising an amount of an antagonist effective to reduce the activity of a GALR2 receptor and a pharmaceutically acceptable carrier.

10   This invention provides a pharmaceutical composition comprising an amount of an agonist effective to increase activity of a GALR2 receptor and a pharmaceutically acceptable carrier.

15   This invention provides a transgenic nonhuman mammal expressing DNA encoding a GALR2 receptor. This invention provides a transgenic nonhuman mammal comprising a homologous recombination knockout of the native GALR2 receptor. This invention provides a transgenic nonhuman
20   mammal whose genome comprises antisense DNA complementary to DNA encoding a GALR2 receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a GALR2 receptor and which hybridizes to mRNA encoding a GALR2 receptor thereby reducing its
25   translation.

This invention also provides a process for determining whether a compound can specifically bind to a GALR2 receptor which comprises contacting a cell transfected
30   with and expressing DNA encoding the GALR2 receptor with the compound under conditions permitting binding of compounds to such receptor, and detecting the presence of any such compound specifically bound to the GALR2 receptor, so as to thereby determine whether the ligand
35   specifically binds to the GALR2 receptor.

This invention provides a process for determining whether

- 11 -

a compound can specifically bind to a GALR2 receptor which comprises preparing a cell extract from cells transfected with and expressing DNA encoding the GALR2 receptor, isolating a membrane fraction from the cell

5   extract, contacting the membrane fraction with the compound under conditions permitting binding of compounds to such receptor, and detecting the presence of the compound specifically bound to the GALR2 receptor, so as to thereby determine whether the compound specifically

10   binds to the GALR2 receptor.

In one embodiment, the GALR2 receptor is a mammalian GALR2 receptor.   In another embodiment, the GALR2 receptor is a rat GALR2 receptor.   In still another

15   embodiment, the GALR2 receptor has substantially the same amino acid sequence encoded by the plasmid K985.   In another embodiment, the GALR2 receptor is a human GALR2 receptor.   In still another embodiment, the GALR2 receptor has substantially the same amino acid sequence

20   as the sequence encoded by plasmid BO29.

This invention provides a process for determining whether a compound is a GALR2 receptor agonist which comprises contacting a cell transfected with and expressing DNA

25   encoding the GALR2 receptor with the compound under conditions permitting the activation of the GALR2 receptor, and detecting an increase in GALR2 receptor activity, so as to thereby determine whether the compound is a GALR2 receptor agonist.

30

This invention provides a process for determining whether a compound is a GALR2 receptor antagonist which comprises contacting a cell transfected with and expressing DNA encoding the GALR2 receptor with the compound in the

35   presence of a known GALR2 receptor agonist, such as galanin, under conditions permitting the activation of the GALR2 receptor, and detecting a decrease in GALR2

- 12 -

receptor activity, so as to thereby determine whether the compound is a GALR2 receptor antagonist.

This invention provides a compound determined by the above-described processes. In one embodiment of the above-described processes, the compound is not previously known. In another embodiment, the compound is not previously known to bind to a GALR2 receptor.

This invention provides a process of screening a plurality of chemical compounds not known to bind to a GALR2 receptor to identify a compound which specifically binds to the GALR2 receptor, which comprises (a) contacting cells transfected with and expressing DNA encoding the GALR2 receptor with a compound known to bind specifically to the GALR2 receptor; (b) contacting the preparation of step (a) with the plurality of compounds not known to bind specifically to the GALR2 receptor, under conditions permitting binding of compounds known to bind the GALR2 receptor; (c) determining whether the binding of the compound known to bind to the GALR2 receptor is reduced in the presence of the compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (d) separately determining the binding to the GALR2 receptor of each compound included in the plurality of compounds, so as to thereby identify the compound which specifically binds to the GALR2 receptor.

This invention provides a method of screening a plurality of chemical compounds not known to activate a GALR2 receptor to identify a compound which activates the GALR2 receptor which comprises (a) contacting cells transfected with and expressing the GALR2 receptor with the plurality of compounds not known to activate the GALR2 receptor, under conditions permitting activation of the GALR2 receptor; (b) determining whether the activity of the

- 13 -

GALR2 receptor is increased in the presence of the compounds; and if so (c) separately determining whether the activation of the GALR2 receptor is increased by each compound included in the plurality of compounds, so as to thereby identify the compound which activates the GALR2 receptor.

This invention provides a method of screening a plurality of chemical compounds not known to inhibit the activation of a GALR2 receptor to identify a compound which inhibits the activation of the GALR2 receptor, which comprises (a) preparing a cell extract from cells transfected with and expressing DNA encoding the GALR2 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the plurality of compounds in the presence of a known GALR2 receptor agonist, under conditions permitting activation of the GALR2 receptor; (b) determining whether the activation of the GALR2 receptor is reduced in the presence of the plurality of compounds, relative to the activation of the GALR2 receptor in the absence of the plurality of compounds; and if so (c) separately determining the inhibition of activation of the GALR2 receptor for each compound included in the plurality of compounds, so as to thereby identify the compound which inhibits the activation of the GALR2 receptor.

This invention provides a method of detecting expression of a GALR2 receptor by detecting the presence of mRNA coding for the GALR2 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with the above-described nucleic acid probe under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the GALR2 receptor by the cell.

This invention provides a method of treating an

- 14 -

abnormality in a subject, wherein the abnormality is
alleviated by the inhibition of a GALR2 receptor which
comprises administering to a subject an effective amount
of the above-described pharmaceutical composition
5   effective to decrease the activity of the GALR2 receptor
in the subject, thereby treating the abnormality in the
subject. In an embodiment, the abnormality is obesity.
In another embodiment, the abnormality is bulimia.

10   This invention provides a method of treating an
abnormality in a subject wherein the abnormality is
alleviated by the activation of a GALR2 receptor which
comprises administering to a subject an effective amount
of the above-described pharmaceutical composition
15   effective to activate the GALR2 receptor in the subject.
In an embodiment, the abnormal condition is anorexia.

This invention provides a method for diagnosing a
predisposition to a disorder associated with the activity
20   of a specific human GALR2 receptor allele which
comprises: (a) obtaining DNA of subjects suffering from
the disorder; (b) performing a restriction digest of the
DNA with a panel of restriction enzymes; (c)
electrophoretically separating the resulting DNA
25   fragments on a sizing gel; (d) contacting the resulting
gel with a nucleic acid probe capable of specifically
hybridizing with a unique sequence included within the
sequence of a nucleic acid molecule encoding a human
GALR2 receptor and labelled with a detectable marker; (e)
30   detecting labelled bands which have hybridized to DNA
encoding a human GALR2 receptor labelled with a
detectable marker to create a unique band pattern
specific to the DNA of subjects suffering from the
disorder; (f) preparing DNA obtained for diagnosis by
35   steps a-e; and (g) comparing the unique band pattern
specific to the DNA of subjects suffering from the
disorder from step e and the DNA obtained for diagnosis

- 15 -

from step f to determine whether the patterns are the
same or different and to diagnose thereby predisposition
to the disorder if the patterns are the same.

5   This invention provides a method of modifying feeding
behavior of a subject which comprises administering to
the subject an amount of a compound which is a galanin
receptor agonist or antagonist effective to increase or
decrease the consumption of food by the subject so as to
10   thereby modify feeding behavior of the subject. In an
embodiment, the compound is a GALR2 receptor antagonist
and the amount is effective to decrease the consumption
of food by the subject. In another embodiment the
compound is administered in combination with food.

15

In yet another embodiment the compound is a GALR2
receptor agonist and the amount is effective to increase
the consumption of food by the subject. In a still
further embodiment, the compound is administered in
20   combination with food. In other embodiments the subject
is a vertebrate, a mammal, a human or a canine.

- 16 -

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 Nucleotide coding sequence of the rat hypothalamic galanin GALR2 receptor (Seq. I.D. No. 7), with partial 5' and 3' untranslated sequences. Start

5 (ATG) and stop (TAA) codons are underlined.

Figure 2 Deduced amino acid sequence of the rat hypothalamic galanin GALR2 receptor encoded by the nucleotide sequence shown in Figure 1 (Seq. I.D. No. 8).

10

Figures 3A-3C 3A. Diagram of the intron-exon arrangement of the rat GALR2 receptor cDNA contained in plasmid K985. Untranslated regions are indicated by dark hatched segments, and coding region is unmarked except for light

15 gray hatched segments indicating the location of the transmembrane domains of the rat GALR2 receptor. The black segment indicates the location of the intron. **3B.** Splice junction sequences of the rat GALR2 receptor. Nucleotide number 1 is located 45 nucleotides upstream of

20 the start codon (Seq. I.D. No. 9). **3C.** Intron sequence of rat GALR2 receptor cDNA contained in plasmid K985. Nucleotide number 1 is located 45 nucleotides upstream of the start codon (Seq. I.D. No. 9).

25 **Figures 4A-4C** Localization of [^{125}I]galanin binding sites in rat CNS. **Figure 4A-1 and 4A-4:** Distribution of total [^{125}I]galanin binding in coronal sections through the hypothalamus and amygdala. **Figures 4A-2 and 4A-5:** Binding which remains in these areas following incubation

30 with 60 nM [D-Trp^2]galanin$_{(1-29)}$. **Figures 4A-3 and 4A-6:** Binding obtained after incubation with 5 μM porcine galanin, which represents the non-specific binding condition. **Figure 4B: Figures 4B-1 to 4B8:** Higher magnification photomicrographs of the [^{125}I]galanin binding

35 sites in the hypothalamus and amygdala. **Figure 4B-1:** Total binding in the paraventricular hypothalamic nucleus (PVN), virtually all of which is removed by 60 nM [D-

- 17 -

Trp²]galanin$_{(1-29)}$(panel 3B).   **Figures 4B-3 and 4B-4:**
Binding in the ventromedial hypothalamus (VMH), lateral
hypothalamus (LH), and zona incerta (ZI). In these
regions,   some   [^{125}I]galanin   binding   remains   after

5   incubation with 60 nM [D-Trp²]galanin$_{(1-29)}$ (Figure 4B-4).
Figures 4B-5 and 4B-7: Total binding in the amygdala.
After incubation with 60 nM [D-Trp²]galanin$_{(1-29)}$ (panels 5B
and 6B), the binding is markedly reduced in the piriform
cortex (Pir), and to a lesser extent in the medial

10   nucleus (Me), and central nucleus (Ce). However, the
binding in the nucleus of the lateral olfactory tract
(LOT) is largely unaffected. **Figure 4C: Panels 4C-1 to
4C-6:** Distribution of [^{125}I]galanin binding sites in the
anterior forebrain (panel 7) and in the midbrain (panel

15   8). In the lateral septum (LS) and insular cortex (CTX),
much of the total binding (panel 7A) is removed by 60 nM
[D-Trp²]galanin$_{(1-29)}$ (panel 7B). Similarly, the total
binding observed in the superior colliculus (SC), central
gray (CG), and pontine reticular nucleus (PnO) (panel 8A)

20   is markedly diminished (panel 8B). **Figures 4C-3 and 4C-
6:** Nonspecific binding observed in adjacent sections
through the septum and midbrain. Arc, arcuate nucleus;.
Ce, central amygdaloid nucleus; CL, centrolateral
thalamic nucleus; LOT, nucleus of the lateral olfactory

25   tract; Me, medial amygdaloid nucleus; Pir, piriform
cortex; PVN, paraventricular hypothalamic nucleus; SO,
supraoptic   nucleus;   st,   stria   terminalis;   VMH,
ventromedial hypothalamic nucleus; ZI, zona incerta.

30   **Figure 5.** Reverse transcriptase PCR (RT-PCR) of rat
GALR2 receptor mRNA from various brain regions. The blot
was hybridized at high stringency with an oligonucleotide
probe corresponding to a portion of the predicted V/VI
loop of GALR2. Positive controls are indicated by +'s and

35   represent plasmids containing the indicated inserts.
Size standards are indicated at the left in kilobases.
Note the additional hybridizing bands intermediate in

                                   - 18 -

size between the intron-containing and the intronless
product.

Figures 6A-6B. Northern blot analysis of GALR2 receptor
mRNA from various rat brain regions.

6A. A Northern blot containing poly A⁺ RNA (~5 μg) from
six different rat brain regions was hybridized at high
stringency with a randomly primed radiolabeled fragment
representing the entire rat GALR2 coding region (not
including the intron). The autoradiogram represents a
four day exposure and reveals a ~1.8-2.0 kb transcript.
6B. The blot was reprobed with 1B15 (~1 kb) to confirm
that similar amounts of RNA were present in each lane.

Figures 7A-7B. Northern blot analysis of GALR2 receptor
mRNA from various rat tissues.

7A. A Northern blot containing poly A⁺ RNA (~2 μg) from
eight different rat tissues was hybridized at high
stringency with a randomly primed radiolabeled fragment
representing the entire rat GALR2 coding region (not
including the intron). The autoradiogram represents a
four day exposure and reveals a single ~ 1.8-2.0 kb
transcript.

7B. The Northern blot was reprobed for 1B15 (~1 kb) to
confirm that similar amounts of RNA were present in each
lane. A second Northern blot (not shown) was also
hybridized under the same conditions and showed similar
results (Table 3).

Figures 8A-8D. Rat GALR2 receptor autoradiography in
COS-7 cells transfected with GALR1 and GALR2 cDNAs. ^{125}I-
[D-Trp^2]Galanin $_{(1-29)}$ was tested as a selective radioligand
for GALR2. Panels represent dark-field photomicrographs
(200X) of photoemulsion-dipped slides. **8A:** Binding of 3
nM ^{125}I-[D-Trp^2]Galanin $_{(1-29)}$ to COS-7 cells transiently
transfected with GALR2. Note positive binding to cells.

8B: Nonspecific binding of 6 nM ^{125}I-[D-Trp^2]Galanin $_{(1-29)}$ in the presence of 300 nM porcine galanin $_{(1-29)}$ to COS-7 cells transiently transfected with GALR2.

8C: Binding of 6 nM ^{125}I-[D-Trp^2]Galanin $_{(1-29)}$ to COS-7 cells transiently transfected with GALR1. Note absence of binding to cell profiles; small accumulations of silver grains represent nonspecific nuclear association. **8D:** Nonspecific binding of 6 nM ^{125}I-[D-Trp^2]Galanin $_{(1-29)}$ in the presence of 600 nM porcine galanin $_{(1-29)}$ to COS-7 cells transiently transfected with GALR1.

Figures 9A-9B. Functional response mediated by LM(tk-) cells stably transfected with the cDNA encoding the rat GALR2 receptor. **9A:** Inhibition of cyclic AMP formation: cells were incubated with varying concentrations of porcine galanin $_{(1-29)}$, and 10 μM forskolin for 15 min. at 37°C Data was normalized taking as 0% the basal levels of cyclic AMP (0.06 ± 0.02 pmol/ml) and 100% the cAMP levels produced by forskolin in the absence of agonist (0.26 ± 0.03 pmol/ml). Data is shown as mean ± standard error of the mean of four independent experiments. **9B:** Phosphoinositide metabolism: cells were incubated for 18 hours in the presence of 0.5 μCi [^{3}H]myo-inositol. Eleven different concentrations of porcine galanin $_{(1-29)}$ were added in the presence on 10mM LiCl. Cells were incubated for 1 hour at 37°C, and [^{3}H]inositol phosphates were isolated and measured.

Figure 10. Nucleotide coding sequence of the human galanin GALR2 receptor (Seq. I.D. No. 29), with partial 5' and 3' untranslated sequences. Start (ATG) and stop (TGA) codons are underlined.

Figure 11. Deduced amino acid sequence of the human galanin GALR2 receptor encoded by the nucleotide sequence shown in Figure 10 (Seq. I.D. No. 30).

Figures 12A-12C.   **12A.** Diagram of the intron-exon arrangement of the human GALR2 receptor cDNA contained in plasmid BO29. Untranslated regions are indicated by dark hatched segments, and coding region is unmarked except for light gray hatched segments indicating the location of the transmembrane domains of the human GALR2 receptor. The black segment indicates the location of the intron. **12B.** Splice junction sequences of the human GALR2 receptor. **12C.** Intron sequence of human GALR2 receptor cDNA contained in plasmid BO29 (Seq. I.D. No. 31).

Figure 13.   Current response in an oocyte injected with 50 pg of GALR2 mRNA. Holding potential was -80 mV.

Figure 14.   Autoradiograph demonstrating hybridization of radiolabeled rGalR2 probe to RNA extracted from rat. The lower band (arrow) represents mRNA coding for the rat GALR2 extracted from tissue indicated at the bottom of the gel. RNA coding for the rat GALR2 is present in: the heart, kidney, hypothalamus, hippocampus, amygdala, spinal cord, and cerebellum. mRNA coding for the rat GALR2 was not detected in RNA extracted from striated muscle or liver.

Figures 15A-15D.   Amino acid sequence alignment of the rat GALR2 receptor (top row) (Seq. ID No. 8), human GALR2 receptor (middle row) (Seq. ID No. 29) and rat GALR1 receptor (bottom row) (Seq. ID No. 32). Transmembrane domains (TM 1-7) are indicated by brackets above the sequence.

- 21 -

DETAILED DESCRIPTION OF THE INVENTION

Throughout this application, the following standard
abbreviations are used to indicate specific nucleotide
bases:

5   C=cytosine   A=adenine
           T=thymine   G=guanine

Furthermore, the term "agonist" is used throughout this
application to indicate any peptide or non-peptidyl
compound which increases the activity of any of the
10   receptors of the subject invention. The term "antagonist"
is used throughout this application to indicate any
peptide or non-peptidyl compound which decreases the
activity of any of the receptors of the subject
invention.

15

The activity of a G-protein coupled receptor such as a
galanin receptor may be measured using any of a variety
of functional assays in which activation of the receptor
in question results in an observable change in the level
20   of some second messenger system, including but not
limited to adenylate cyclase, calcium mobilization,
arachidonic acid release, ion channel activity, inositol
phospholipid hydrolysis or guanylyl cyclase.
Heterologous expression systems utilizing appropriate
25   host cells to express the nucleic acid of the subject
invention are used to obtain the desired second messenger
coupling. Receptor activity may also be assayed in an
oocyte expression system.

30   This invention provides an isolated nucleic acid encoding
a GALR2 galanin receptor. In an embodiment, the galanin
receptor is a vertebrate or a mammalian GALR2 receptor.
In another embodiment, the galanin receptor is a rat
GALR2 receptor. In another embodiment, the galanin
35   receptor is a human GALR2 receptor. In an embodiment,
the isolated nucleic acid encodes a receptor
characterized by an amino acid sequence in the

- 22 -

transmembrane region, which has a homology of 60% or
higher to the amino acid sequence in the transmembrane
region of the rat galanin GALR2 receptor and a homology
of less than 60% to the amino acid sequence in the
5   transmembrane region of any GALR1 receptor.   In one
embodiment, the GALR2 receptor is a rat GALR2 receptor.
In another embodiment, the GALR2 receptor is a human
GALR2 receptor.

10   This invention provides an isolated nucleic acid encoding
a GALR2 receptor having substantially the same amino acid
sequence as shown in Figure 2.   In one embodiment, the
nucleic acid is DNA.   This invention further provides an
isolated nucleic acid encoding a rat GALR2 receptor
15   having the amino acid sequence shown in Figure 2.   In
another embodiment, the nucleic acid comprises at least
an intron.   In yet another embodiment, the intron
comprises a fragment of the intron sequence shown in
Figure 3C (Seq. I.D. No. 9).   In still another
20   embodiment, the nucleic acid comprises alternately
spliced nucleic acid transcribed from the nucleic acid
contained in plasmid K985.   In one embodiment, the
alternately spliced nucleic acid is mRNA transcribed from
DNA encoding a galanin receptor.

25

In one embodiment, the GALR2 receptor has substantially
the same amino acid sequence as the amino acid sequence
encoded by plasmid K985 (ATCC Accession No. 97426).   In
another embodiment, the GALR2 receptor has the amino
30   acid sequence encoded by the plasmid K985.   In still
another embodiment, the GALR2 receptor has substantially
the same amino acid sequence as the amino acid sequence
encoded by the plasmid K1045. In yet another embodiment,
the GALR2 receptor has the amino acid sequence encoded by
35   the plasmid K1045. Plasmid K1045 comprises an intronless
cDNA encoding the rat GALR2 receptor. Plasmid K1045 is
further characterized by its lack of native 5' or 3'

- 23 -

untranslated sequences, such that the plasmid contains
only the regulatory elements necessary for expression in
mammalian cells (e.g., Kozak consensus sequence) and the
coding sequence of the GALR2 receptor.

5

In one embodiment, the human GALR2 receptor has
substantially the same amino acid sequence as the amino
acid sequence encoded by plasmid BO29 (ATCC Accession No.
97735). In yet another embodiment, the human GALR2
10   receptor has the amino acid sequence encoded by the
plasmid BO29. In another embodiment, the nucleic acid
encoding the human GALR2 receptor comprises an intron.
In still another embodiment, the nucleic acid encoding
the human GALR2 receptor comprises alternately spliced
15   nucleic acid transcribed from the nucleic acid contained
in plasmid BO29. In still another embodiment, the human
GALR2 receptor has substantially the same amino acid
sequence as the amino acid sequence encoded by plasmid
BO39 (ATCC Accession No.   ). In another embodiment,
20   the human GALR2 receptor has the amino acid sequence
encoded by the plasmid BO39. Plasmid BO39 comprises an
intronless cDNA encoding the human GALR2 receptor. This
invention provides an isolated nucleic acid encoding a
GALR2 receptor having substantially the same amino acid
25   sequence as shown in Figure 11 (Seq. I.D. No. 30). In
one embodiment, the nucleic acid is DNA. This invention
further provides an isolated nucleic acid encoding a
human GALR2 receptor having the amino acid sequence shown
in Figure 11.

30

The observation that both the human and rat GALR2 cDNAs
contain at least one intron raises the possibility that
additional introns could exist in coding or non-coding
regions. In addition, spliced form(s) of mRNA may encode
35   additional amino acids either upstream of the currently
defined starting methionine or within the coding region.
Further, the existence and use of alternative exons is

- 24 -

possible, whereby the mRNA may encode different amino
acids within the region comprising the exon.   In
addition, single amino acid substitutions may arise via
the mechanism of RNA editing such that the amino acid

5   sequence of the expressed protein is different than that
encoded by the original gene (Burns et al., 1996; Chu et
al., 1996).   Such variants may exhibit pharmacologic
properties differing from the receptor encoded by the
original gene.

10

This invention provides a splice variant of the GALR2
receptors disclosed herein.   This invention further
provides for alternate translation initiation sites and
alternately spliced or edited variants of nucleic acids

15   encoding rat and human GALR2 receptors.

This invention provides the above-described isolated
nucleic acid, wherein the nucleic acid is DNA.   In an
embodiment, the DNA is cDNA.   In another embodiment, the

20   DNA is genomic DNA.   In still another embodiment, the
nucleic acid molecule is RNA.   Methods for production and
manipulation of nucleic acid molecules are well known in
the art.

25   In another embodiment, the nucleic acid encodes a
vertebrate GALR2 receptor.   In a separate embodiment, the
nucleic acid encodes a mammalian GALR2 receptor.   In
another embodiment, the nucleic acid encodes a rat GALR2
receptor.   In still another embodiment, the nucleic acid

30   encodes a human GALR2 receptor.

This invention further provides nucleic acid which is
degenerate with respect to the DNA comprising the coding
sequence of the plasmid K985.   This invention also

35   provides nucleic acid which is degenerate with respect to
the DNA comprising the coding sequence of the plasmid
K1045.   This invention further provides nucleic acid

which is degenerate with respect to any DNA encoding a
GALR2 receptor. In one embodiment, the nucleic acid
comprises a nucleotide sequence which is degenerate with
respect to the nucleotide sequence described in Figure 1
5   (Seq. I.D. No. 1), that is, a nucleotide sequence which
is translated into the same amino acid sequence.   In
another embodiment, the nucleic acid comprises a
nucleotide sequence which is degenerate with respect to
the nucleotide sequence described in Seq. I.D. No. 9.

10

In yet another embodiment, this invention further
provides nucleic acid which is degenerate with respect to
the DNA comprising the coding sequence of plasmid BO29.
In an embodiment, the nucleic acid comprises a nucleotide

15   sequence which is degenerate with respect to the
nucleotide sequence described in Figure 10 (Seq. I.D. No.
29), that is, a nucleotide sequence which is translated
into the same amino acid sequence. This invention also
provides nucleic acid which is degenerate with respect to

20   the DNA comprising the coding sequence of the plasmid
BO39.

This invention also encompasses DNAs and cDNAs which
encode amino acid sequences which differ from those of

25   the GALR2 galanin receptor, but which should not produce
phenotypic changes. Alternatively, this invention also
encompasses DNAs, cDNAs, and RNAs which hybridize to the
DNA, cDNA, and RNA of the subject invention.
Hybridization methods are well known to those of skill in

30   the art.

The nucleic acids of the subject invention also include
nucleic acid molecules coding for polypeptide analogs,
fragments or derivatives of antigenic polypeptides which

35   differ from naturally-occurring forms in terms of the
identity or location of one or more amino acid residues
(deletion analogs containing less than all of the

- 26 -

residues specified for the protein, substitution analogs
wherein one or more residues specified are replaced by
other residues and addition analogs where in one or more
amino acid residues is added to a terminal or medial
portion of the polypeptides) and which share some or all
properties of naturally-occurring forms. These molecules
include: the incorporation of codons "preferred" for
expression by selected non-mammalian hosts; the provision
of sites for cleavage by restriction endonuclease
enzymes; and the provision of additional initial,
terminal or intermediate DNA sequences that facilitate
construction of readily expressed vectors.

G-protein coupled receptors such as the GALR2 receptors
of the present invention are characterized by the ability
of an agonist to promote the formation of a high-affinity
ternary complex between the agonist, the receptor, and an
intracellular G-protein. This complex is formed in the
presence of physiological concentrations of GTP, and
results in the dissociation of the alpha subunit of the
G protein from the beta and gamma subunits of the G
protein, which further results in a functional response,
i.e., activation of downstream effectors such as adenylyl
cyclase or phospholipase C. This high-affinity complex
is transient even in the presence of GTP, so that if the
complex is destablized, the affinity of the receptor for
agonists is reduced. Thus, if a receptor is not
optimally coupled to G protein under the conditions of an
assay, an agonist will bind to the receptor with low
affinity. In contrast, the affinity of the receptor for
an antagonist is normally not significantly affected by
the presence or absence of G protein. Functional assays
may be used to determine whether a compound binds to the
receptor, but may be more time-consuming or difficult to
perform than a binding assay. Therefore, it may
desirable to produce a receptor which will bind to
agonists with high affinity in a binding assay. Examples

of modified receptors which bind agonists with high
affinity are disclosed in WO 96/14331, which describes
neuropeptide Y receptors modified in the third
intracellular domain. The modifications may include
deletions of 6-13 amino acids in the third intracellular
loop. Such deletions preferaby end immediately before
the polar or charged residue at the beginning of helix
six. In one embodiment, the deleted amino acids are at
the carboxy terminal portion of the third intracellular
domain. Such modified receptors may be produced using
methods well-known in the art such as site-directed
mutagenesis or recombinant techniques using restriction
enzymes.

This invention provides an isolated nucleic acid encoding
a modified GALR2 receptor, which differs from a GALR2
receptor by having an amino acid(s) deletion, replacement
or addition in the third intracellular domain. In one
embodiment, the modified GALR2 receptor differs by having
a deletion in the third intracellular domain. In another
embodiment, the modified GALR2 receptor differs by having
an amino acid replacement or addition to the third
intracellular domain.

The modified receptors of this invention may be
transfected into cells either transiently or stably using
methods well-known in the art, examples of which are
disclosed herein. This invention also provides for
binding assays using the modified receptors, in which the
receptor is expressed either transiently or in stable
cell lines. This invention further provides for a
compound identified using a modified receptor in a
binding assay such as the binding assays described
herein.

The nucleic acids described and claimed herein are useful
for the information which they provide concerning the

- 28 -

amino acid sequence of the polypeptide and as products for the large scale synthesis of the polypeptide by a variety of recombinant techniques. The nucleic acid molecule is useful for generating new cloning and

5   expression vectors, transformed and transfected prokaryotic and eukaryotic host cells, and new and useful methods for cultured growth of such host cells capable of expression of the polypeptide and related products.

10   This invention also provides an isolated galanin GALR2 receptor protein. In one embodiment, the GALR2 receptor protein has the same or substantially the same amino acid sequence as the amino acid sequence encoded by plasmid K985. In another embodiment, the GALR2 receptor protein

15   has the same or substantially the same amino acid sequence as the amino acid sequence encoded by plasmid K1045. In one embodiment, the GALR2 receptor protein has the same or substantially the same amino acid sequence as shown in Figure 2. In another embodiment, the GALR2

20   receptor has the amino acid sequence shown in Figure 2. In still another embodiment, the GALR2 receptor protein has the same or substantially the same amino acid sequence as the amino acid sequence encoded by plasmid BO29. In still another embodiment, the GALR2 receptor

25   protein has the same or substantially the same amino acid sequence as the amino acid sequence encoded by plasmid BO39. In an embodiment, the GALR2 receptor protein has the same or substantially the same amino acid sequence as shown in Figure 11. In another embodiment, the GALR2

30   receptor has the amino acid sequence shown in Figure 11.

This invention provides a vector comprising the above-described nucleic acid molecule.

35   Vectors which comprise the isolated nucleic acid molecule described hereinabove also are provided. Suitable vectors comprise, but are not limited to, a plasmid or a

virus. These vectors may be transformed into a suitable
host cell to form a host cell expression system for the
production of a polypeptide having the biological
activity of a galanin GALR2 receptor. Suitable host
5   cells include, for example, neuronal cells such as the
glial cell line C6, a Xenopus cell such as an oocyte or
melanophore cell, as well as numerous mammalian cells and
non-neuronal cells.

10   This invention provides the above-described vector
adapted for expression in a bacterial cell which further
comprises the regulatory elements necessary for
expression of the nucleic acid in the bacterial cell
operatively linked to the nucleic acid encoding the GALR2
15   receptor as to permit expression thereof.

This invention provides the above-described vector
adapted for expression in a yeast cell which comprises
the regulatory elements necessary for expression of the
20   nucleic acid in the yeast cell operatively linked to the
nucleic acid encoding the GALR2 receptor as to permit
expression thereof.

This invention provides the above-described vector
25   adapted for expression in an insect cell which comprises
the regulatory elements necessary for expression of the
nucleic acid in the insect cell operatively linked to the
nucleic acid encoding the GALR2 receptor as to permit
expression thereof. In a still further embodiment, the
30   vector is a baculovirus.

In one embodiment, the vector is adapted for expression
in a mammalian cell which comprises the regulatory
elements necessary for expression of the nucleic acid in
35   the mammalian cell operatively linked to the nucleic acid
encoding the mammalian GALR2 receptor as to permit
expression thereof.

- 30 -

In a further embodiment, the vector is adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding the rat GALR2 receptor as to permit expression thereof.

In a still further embodiment, the vector is a plasmid.

In another embodiment, the plasmid is adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding the human GALR2 receptor as to permit expression thereof.

This invention provides the above-described plasmid adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of nucleic acid in a mammalian cell operatively linked to the nucleic acid encoding the mammalian GALR2 receptor as to permit expression thereof.

This invention provides a plasmid designated K985 (ATCC Accession No. 97426) which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to DNA encoding the GALR2 galanin receptor so as to permit expression thereof.

This plasmid (K985) was deposited on January 24, 1996, with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 97426.

- 31 -

This invention provides a plasmid designated BO29 (ATCC Accession No. 97735) which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to DNA encoding the GALR2 galanin

5   receptor as to permit expression thereof.

This plasmid (BO29) was deposited on September 25, 1996, with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under

10   the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 97735.

15   This invention provides a plasmid designated K1045 (ATCC Accession No. 97778) which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to DNA encoding the GALR2 galanin receptor so as to permit expression thereof.

20
This plasmid (K1045) was deposited on October 30, 1996, with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the

25   International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 97426.

This invention provides a plasmid designated BO39 (ATCC

30   Accession No. _____) which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to DNA encoding the GALR2 galanin receptor as to permit expression thereof.

35   This plasmid (BO39) was deposited on January 15, 1997, with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under

the provisions of the Budapest Treaty for the
International Recognition of the Deposit of
Microorganisms for the Purposes of Patent Procedure and
was accorded ATCC Accession No. _____.

This invention further provides for any vector or plasmid
which comprises modified untranslated sequences, which
are beneficial for expression in desired host cells or
for use in binding or functional assays. For example, a
vector or plasmid with untranslated sequences of varying
lengths may express differing amounts of the receptor
depending upon the host cell used. In an embodiment, the
vector or plasmid comprises the coding sequence of the
GALR2 receptor and the regulatory elements necessary for
expression in the host cell.

This invention provides a eukaryotic cell comprising the
above-described plasmid or vector. This invention
provides a mammalian cell comprising the above-described
plasmid or vector. In an embodiment the cell is a
Xenopus oocyte or melanophore cell. In an embodiment,
the cell is a neuronal cell such as the glial cell line
C6. In an embodiment, the mammalian cell is non-neuronal
in origin. In an embodiment, the mammalian cell is a
COS-7 cell. In another embodiment the mammalian cell is
a Chinese hamster ovary (CHO) cell. In another
embodiment, the cell is a mouse Y1 cell.

In still another embodiment, the mammalian cell is a 293
human embryonic kidney cell. In still another
embodiment, the mammalian cell is a NIH-3T3 cell. In
another embodiment, the mammalian cell is an LM(tk-)
cell. In still another embodiment, the mammalian cell is
the LM(tk-) cell designated L-rGALR2-8. This cell line
was deposited with the ATCC on March 28, 1996, under the
provisions of the Budapest Treaty for the International
Recognition of the Deposit of Microorganisms for the

- 33 -

Purposes of Patent Procedure, and was accorded ATCC
Accession No. CRL-12074. In yet another embodiment, the
mammalian cell is the LM(tk-) cell designated L-rGALR2I-4
(which comprises the intronless plasmid K1045). This

5   cell line was deposited with the ATCC on October 30,
1996, under the provisions of the Budapest Treaty for the
International Recognition of the Deposit of
Microorganisms for the Purposes of Patent Procedure, and
was accorded ATCC Accession No. CRL-12223.

10

In another embodiment, the mammalian cell is the Chinese
hamster ovary (CHO) cell designated C-rGalR2-79. C-
rGalR2-79 expresses the rat GALR2 receptor and comprises
a plasmid containing the intron within the coding region.

15   This cell line was deposited with the ATCC on January 15,
1997, under the provisions of the Budapest Treaty for the
International Recognition of the Deposit of
Microorganisms for the Purposes of Patent Procedure, and
was accorded ATCC Accession No. CRL-12262.

20

This invention also provides an insect cell comprising
the above-described vector. In an embodiment, the insect
cell is an Sf9 cell. In another embodiment, the insect
cell is an Sf21 cell.

25

This invention provides a membrane preparation isolated
from any of the above-described cells.

This invention provides a nucleic acid probe comprising

30   at least 15 nucleotides, which probe specifically
hybridizes with a nucleic acid encoding a GALR2 receptor,
wherein the probe has a unique sequence corresponding to
a sequence present within one of the two strands of the
nucleic acid encoding the GALR2 receptor contained in

35   plasmid K985.

This invention further provides a nucleic acid probe

- 34 -

comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a GALR2 receptor, wherein the probe has a unique sequence corresponding to a sequence present within one of the two
5   strands of the nucleic acid encoding the GALR2 receptor contained in plasmid K1045.

This invention still further provides a nucleic acid probe comprising at least 15 nucleotides, which probe
10   specifically hybridizes with a nucleic acid encoding a GALR2 receptor, wherein the probe has a unique sequence corresponding to a sequence present within (a) the nucleic acid sequence described in Figure 1 or (b) the reverse complement thereto. This invention also provides
15   a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a GALR2 receptor, wherein the probe has a unique sequence corresponding to a sequence present within one of the two strands of the nucleic acid encoding the GALR2
20   receptor contained in plasmid BO29. This invention also provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a GALR2 receptor, wherein the probe has a unique sequence corresponding to a sequence present
25   within one of the two strands of the nucleic acid encoding the GALR2 receptor contained in plasmid BO39.

This invention provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically
30   hybridizes with a nucleic acid encoding a GALR2 receptor, wherein the probe has a unique sequence corresponding to a sequence present within (a) the nucleic acid sequence shown in Figure 10 (Seq. ID No. 29) or (b) the reverse complement to the nucleic acid sequence shown in Figure
35   10.

This invention provides a nucleic acid probe comprising

at least 15 nucleotides, which probe specifically
hybridizes with a nucleic acid encoding a GALR2 receptor,
wherein the probe has a unique sequence corresponding to
a sequence present within (a) the nucleic acid sequence

5   shown in Figure 1 (Seq. I.D. No. 7) or (b) the reverse
complement to the nucleic acid sequence shown in Figure
1 (Seq. I.D. No. 7). In one embodiment, the nucleic acid
encoding a GALR2 receptor comprises an intron, the
sequence of which intron is described in Figure 3 (Seq.

10   I.D. No. 9). In another embodiment, the nucleic acid
encoding a GALR2 receptor comprises an intron, the
sequence of which intron is described in Figure 12C (Seq.
I.D. No. 31).

15   This invention further provides a nucleic acid probe
comprising a nucleic acid molecule of at least 15
nucleotides which is complementary to a unique fragment
of the sequence of a nucleic acid molecule encoding a
GALR2 receptor. This invention also provides a nucleic

20   acid probe comprising a nucleic acid molecule of at least
15 nucleotides which is complementary to the antisense
sequence of a unique fragment of the sequence of a
nucleic acid molecule encoding a GALR2 receptor.

25   In one embodiment, the nucleic acid probe is DNA. In
another embodiment the nucleic acid probe is RNA. As
used herein, the phrase "specifically hybridizing" means
the ability of a nucleic acid molecule to recognize a
nucleic acid sequence complementary to its own and to

30   form double-helical segments through hydrogen bonding
between complementary base pairs.

This nucleic acid of at least 15 nucleotides capable of
specifically hybridizing with a sequence of a nucleic

35   acid encoding the GALR2 galanin receptors can be used as
a probe. Nucleic acid probe technology is well known to
those skilled in the art who will readily appreciate that

such probes may vary greatly in length and may be labeled
with a detectable label, such as a radioisotope or
fluorescent dye, to facilitate detection of the probe.
DNA probe molecules may be produced by insertion of a DNA
5   molecule which encodes the GALR2 receptor into suitable
vectors, such as plasmids or bacteriophages, followed by
transforming into suitable bacterial host cells,
replication in the transformed bacterial host cells and
harvesting of the DNA probes, using methods well known in
10   the art.   Alternatively,   probes   may   be   generated
chemically from DNA synthesizers.

RNA probes may be generated by inserting the DNA molecule
which encodes the GALR2 galanin receptor downstream of a
15   bacteriophage promoter such as T3, T7 or SP6.   Large
amounts of RNA probe may be produced by incubating the
labeled nucleotides with the linearized fragment where it
contains an upstream promoter in the presence of the
appropriate RNA polymerase.
20

This invention provides an antisense oligonucleotide
having a sequence capable of specifically hybridizing to
mRNA encoding a GALR2 galanin receptor, so as to prevent
translation of the mRNA.
25

This invention provides an antisense oligonucleotide
having a sequence capable of specifically hybridizing to
the genomic DNA molecule encoding a GALR2 receptor.

30   This invention provides an antisense oligonucleotide
comprising chemical analogues of nucleotides.

This invention provides an antibody directed to a GALR2
receptor.   This invention also provides an antibody
35   directed to a rat GALR2 receptor.   This invention also
provides an antibody directed to a human GALR2 receptor.
In an embodiment, the human GALR2 has an amino acid

- 37 -

sequence the same or substantially the same as an amino acid sequence encoded by plasmid K985 or an amino acid sequence encoded by plasmid BO29. In another embodiment, the human GALR2 has an amino acid sequence the same or

5   substantially the same as an amino acid sequence encoded by plasmid BO39.

This invention provides a monoclonal antibody directed to an epitope of a GALR2 receptor, which epitope is present

10   on the surface of a cell expressing a GALR2 receptor.

This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide effective to reduce activity of a GALR2 receptor by passing through a

15   cell membrane and binding specifically with mRNA encoding a GALR2 receptor in the cell so as to prevent its translation and a pharmaceutically acceptable carrier capable of passing through a cell membrane. In one embodiment, the oligonucleotide is coupled to a substance

20   which inactivates mRNA. In another embodiment, the substance which inactivates mRNA is a ribozyme.

This invention provides the above-described pharmaceutical composition, wherein the pharmaceutically

25   acceptable carrier capable of passing through a cell membrane comprises a structure which binds to a receptor specific for a selected cell type and is thereby taken up by cells of the selected cell type.

30   This invention provides a pharmaceutical composition comprising an amount of an antagonist effective to reduce the activity of a GALR2 receptor and a pharmaceutically acceptable carrier.

35   This invention provides a pharmaceutical composition comprising an amount of an agonist effective to increase activity of a GALR2 receptor and a pharmaceutically

- 38 -

acceptable carrier.

This invention provides the above-described pharmaceutical composition which comprises an amount of the antibody effective to block binding of a ligand to the GALR2 receptor and a pharmaceutically acceptable carrier.

As used herein, "pharmaceutically acceptable carriers" means any of the standard pharmaceutically acceptable carriers. Examples include, but are not limited to, phosphate buffered saline, physiological saline, water and emulsions, such as oil/water emulsions.

This invention provides a transgenic nonhuman mammal expressing DNA encoding a GALR2 receptor.

This invention provides a transgenic nonhuman mammal comprising a homologous recombination knockout of the native GALR2 receptor.

This invention provides a transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a GALR2 receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a GALR2 receptor and which hybridizes to mRNA encoding a GALR2 receptor thereby reducing its translation.

This invention provides the above-described transgenic nonhuman mammal, wherein the DNA encoding a GALR2 receptor additionally comprises an inducible promoter.

This invention provides the transgenic nonhuman mammal, wherein the DNA encoding a GALR2 receptor additionally comprises tissue specific regulatory elements.

In an embodiment, the transgenic nonhuman mammal is a
mouse.

Animal model systems which elucidate the physiological
and behavioral roles of GALR2 receptor are produced by
creating transgenic animals in which the activity of the
GALR2 receptor is either increased or decreased, or the
amino acid sequence of the expressed GALR2 receptor is
altered, by a variety of techniques. Examples of these
techniques include, but are not limited to: 1) Insertion
of normal or mutant versions of DNA encoding a GALR2
receptor, by microinjection, electroporation, retroviral
transfection or other means well known to those skilled
in the art, into appropriate fertilized embryos in order
to produce a transgenic animal or 2) Homologous
recombination of mutant or normal, human or animal
versions of these genes with the native gene locus in
transgenic animals to alter the regulation of expression
or the structure of these GALR2 receptor sequences. The
technique of homologous recombination is well known in
the art. It replaces the native gene with the inserted
gene and so is useful for producing an animal that cannot
express native GALR2 receptors but does express, for
example, an inserted mutant GALR2 receptor, which has
replaced the native GALR2 receptor in the animal's genome
by recombination, resulting in underexpression of the
transporter. Microinjection adds genes to the genome,
but does not remove them, and so is useful for producing
an animal which expresses its own and added GALR2
receptors, resulting in overexpression of the GALR2
receptors.

One means available for producing a transgenic animal,
with a mouse as an example, is as follows: Female mice
are mated, and the resulting fertilized eggs are
dissected out of their oviducts. The eggs are stored in
an appropriate medium such as M2 medium. DNA or cDNA

encoding a GALR2 receptor is purified from a vector by
methods well known in the art. Inducible promoters may
be fused with the coding region of the DNA to provide an
experimental means to regulate expression of the trans-
5   gene. Alternatively, or in addition, tissue specific
regulatory elements may be fused with the coding region
to permit tissue-specific expression of the trans-gene.
The DNA, in an appropriately buffered solution, is put
into a microinjection needle (which may be made from
10   capillary tubing using a pipet puller) and the egg to be
injected is put in a depression slide. The needle is
inserted into the pronucleus of the egg, and the DNA
solution is injected. The injected egg is then
transferred into the oviduct of a pseudopregnant mouse (a
15   mouse stimulated by the appropriate hormones to maintain
pregnancy but which is not actually pregnant), where it
proceeds to the uterus, implants, and develops to term.
As noted above, microinjection is not the only method for
inserting DNA into the egg cell, and is used here only
20   for exemplary purposes.

This invention provides a process for identifying a
chemical compound which specifically binds to a GALR2
receptor which comprises contacting cells containing DNA
25   encoding and expressing on their cell surface the GALR2
receptor, wherein such cells do not normally express the
GALR2 receptor, with the compound under conditions
suitable for binding, and detecting specific binding of
the chemical compound to the GALR2 receptor.

30

This invention further provides a process for identifying
a chemical compound which specifically binds to a GALR2
receptor which comprises contacting a membrane fraction
from a cell extract of cells containing DNA encoding and
35   expressing on their cell surface the GALR2 receptor,
wherein such cells do not normally express the GALR2
receptor, with the compound under conditions suitable for

- 41 -

binding, and detecting specific binding of the chemical compound to the GALR2 receptor.

This invention also provides a method for determining
whether a chemical compound can specifically bind to a GALR2 receptor which comprises contacting cells transfected with and expressing DNA encoding the GALR2 receptor with the compound under conditions permitting binding of compounds to such receptor, and detecting the
presence of any such compound specifically bound to the GALR2 receptor, so as to thereby determine whether the ligand specifically binds to the GALR2 receptor.

This invention provides a method for determining whether
a chemical compound can specifically bind to a GALR2 receptor which comprises preparing a cell extract from cells transfected with and expressing DNA encoding the GALR2 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the
compound under conditions permitting binding of compounds to such receptor, and detecting the presence of the compound specifically bound to the GALR2 receptor, so as to thereby determine whether the compound specifically binds to the GALR2 receptor.

In one embodiment, the GALR2 receptor is a mammalian GALR2 receptor. In another embodiment, the GALR2 receptor is a rat GALR2 receptor. In still another embodiment, the GALR2 receptor has the same or
substantially the same amino acid sequence as that encoded by plasmid K985, or plasmid K1045. In still another embodiment, the GALR2 receptor has the same or substantially the same amino acid sequence as the amino acid sequence shown in Figure 2 (Seq. I.D. No.8). In yet
another embodiment, the GALR2 receptor has the amino acid sequence shown in Figure 2 (Seq. I.D. No. 8).

- 42 -

In another embodiment, the GALR2 receptor is a human
GALR2 receptor. In still another embodiment, the human
GALR2 receptor has the same or substantially the same
amino acid sequence as the amino acid sequence encoded by

5   plasmid BO29 or plasmid BO39. In yet another embodiment,
the GALR2 receptor has the same or substantially the same
amino acid sequence as the amino acid sequence shown in
Figure 11 (Seq. I.D. No. 30). In another embodiment, the
GALR2 receptor has the amino acid sequence shown in

10   Figure 11 (Seq. I.D. No. 30).

In one embodiment, the above process further comprises
determining whether the compound selectively binds to the
GALR2 receptor relative to another galanin receptor. In

15   another embodiment, the determination whether the
compound selectively binds to the GALR2 receptor
comprises: (a) determining the binding affinity of the
compound for the GALR2 receptor and for such other
galanin receptor; and (b) comparing the binding

20   affinities so determined, the presence of a higher
binding affinity for the GALR2 receptor than for such
other galanin receptor inicating that the compound
selectively binds to the GALR2 receptor. In an
embodiment, the other galanin receptor is a GALR1

25   receptor. In another embodiment, the other galanin
receptor is a GALR3 receptor.

This invention provides a process for determining whether
a chemical compound is a GALR2 receptor agonist which

30   comprises contacting cells transfected with and
expressing DNA encoding the GALR2 receptor with the
compound under conditions permitting the activation of
the GALR2 receptor, and detecting an increase in GALR2
receptor activity, so as to thereby determine whether the

35   compound is a GALR2 receptor agonist.

This invention provides a process for determining whether

a chemical compound is a GALR2 receptor agonist which
comprises preparing a cell extract from cells transfected
with and expressing DNA encoding the GALR2 receptor,
isolating a membrane fraction from the cell extract,
5   contacting the membrane fraction with the compound under
conditions permitting the activation of the GALR2
receptor, and detecting an increase in GALR2 receptor
activity, so as to thereby determine whether the compound
is a GALR2 receptor agonist.

10

In one embodiment, the GALR2 receptor is a mammalian
GALR2 receptor. In another embodiment, the GALR2
receptor is a rat GALR2 receptor. In still another
embodiment, the GALR2 receptor has the same or
15   substantially the same amino acid sequence as that
encoded by plasmid K985, or plasmid K1045. In still
another embodiment, the GALR2 receptor has the same or
substantially the same amino acid sequence as the amino
acid sequence shown in Figure 2 (Seq. I.D. No.8). In yet
20   another embodiment, the GALR2 receptor has the amino acid
sequence shown in Figure 2 (Seq. I.D. No. 8).

In another embodiment, the GALR2 receptor is a human
GALR2 receptor. In still another embodiment, the human
25   GALR2 receptor has the same or substantially the same
amino acid sequence as the amino acid sequence encoded by
plasmid BO29 or plasmid BO39. In yet another embodiment,
the GALR2 receptor has the same or substantially the same
amino acid sequence as the amino acid sequence shown in
30   Figure 11 (Seq. I.D. No. 30). In another embodiment, the
GALR2 receptor has the amino acid sequence shown in
Figure 11 (Seq. I.D. No. 30).

This invention provides a process for determining whether
35   a chemical compound is a GALR2 receptor antagonist which
comprises contacting cells transfected with and
expressing DNA encoding the GALR2 receptor with the

compound in the presence of a known GALR2 receptor
agonist, such as galanin, under conditions permitting the
activation of the GALR2 receptor, and detecting a
decrease in GALR2 receptor activity, so as to thereby

5   determine whether the compound is a GALR2 receptor
antagonist.

This invention provides a process for determining whether
a chemical compound is a GALR2 receptor antagonist which

10   comprises preparing a cell extract from cells transfected
with and expressing DNA encoding the GALR2 receptor,
isolating a membrane fraction from the cell extract,
contacting the membrane fraction with the ligand in the
presence of a known GALR2 receptor agonist, such as

15   galanin, under conditions permitting the activation of
the GALR2 receptor, and detecting a decrease in GALR2
receptor activity, so as to thereby determine whether the
compound is a GALR2 receptor antagonist.

20   In one embodiment, the GALR2 receptor is a mammalian
GALR2 receptor. In another embodiment, the GALR2
receptor is a rat GALR2 receptor. In still another
embodiment, the GALR2 receptor has the same or
substantially the same amino acid sequence as that

25   encoded by plasmid K985, or plasmid K1045. In still
another embodiment, the GALR2 receptor has the same or
substantially the same amino acid sequence as the amino
acid sequence shown in Figure 2 (Seq. I.D. No.8). In yet
another embodiment, the GALR2 receptor has the amino acid

30   sequence shown in Figure 2 (Seq. I.D. No. 8).

In another embodiment, the GALR2 receptor is a human
GALR2 receptor. In still another embodiment, the human
GALR2 receptor has the same or substantially the same

35   amino acid sequence as the amino acid sequence encoded by
plasmid BO29 or plasmid BO39. In yet another embodiment,
the GALR2 receptor has the same or substantially the same

- 45 -

amino acid sequence as the amino acid sequence shown in
Figure 11 (Seq. I.D. No. 30). In another embodiment, the
GALR2 receptor has the amino acid sequence shown in
Figure 11 (Seq. I.D. No. 30).

5

In an embodiment of the above-described methods, the cell
is an insect cell. In another embodiment, the cell is a
mammalian cell. In a further embodiment, the cell is
non-neuronal in origin. In still further embodiments,
10   the non-neuronal cell is a COS-7 cell, 293 human
embryonic kidney cell, NIH-3T3 cell, a CHO cell, or
LM(tk-) cell. In yet another embodiment of any of the
processes of this invention the cell is the LM(tk-) cell
L-rGALR2-8 (ATCC Accession No. CRL-12074), the LM(tk-)
15   cell L-rGALR2I-4 (ATCC Accession No. CRL-12223, or the
CHO cell C-rGalR2-79 (ATCC Accession No. CRL-12262).

This invention provides a compound determined by the
above-described processes. In one embodiment of the
20   above-described processes, the compound is not previously
known to bind to a GALR2 receptor.

This invention provides a GALR2 agonist determined by the
above-described processes. This invention also provides
25   a GALR2 antagonist determined by the above-described
processes.

This invention provides a pharmaceutical composition
which comprises an amount of a GALR2 receptor agonist
30   effective to increase activity of a GALR2 receptor and a
pharmaceutically acceptable carrier.

This invention provides a pharmaceutical composition
which comprises an amount of a GALR2 receptor antagonist
35   effective to reduce activity of a GALR2 receptor and a
pharmaceutically acceptable carrier.

- 46 -

In further embodiments of the above-described processes, the agonist or antagonist is not previously known to bind to a GALR2 receptor.

5   This invention provides a process involving competitive binding for identifying a chemical compound which specifically binds to a GALR2 receptor, which comprises separately contacting cells expressing on their cell surface the GALR2 receptor, wherein such cells do not
10   normally express the GALR2 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical
15   compound to the GALR2 receptor, a decrease in the binding of the second chemical compound to the GALR2 receptor in the presence of the chemical compound indicating that the chemical compound binds to the GALR2 receptor.

20   This invention further provides a process involving competitive binding for identifying a chemical compound which specifically binds to a human GALR2 receptor, which comprises separately contacting a membrane fraction from a cell extract of cells expressing on their cell surface
25   the GALR2 receptor, wherein such cells do not normally express the GALR2 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds,
30   and detecting specific binding of the chemical compound to the GALR2 receptor, a decrease in the binding of the second chemical compound to the GALR2 receptor in the presence of the chemical compound indicating that the chemical compound binds to the GALR2 receptor.

35

This invention further provides a process for determining whether a chemical compound specifically binds to and

activates a GALR2 receptor, which comprises contacting
cells producing a second messenger response and
expressing on their cell surface the GALR2 receptor,
wherein such cells do not normally express the GALR2

5   receptor, with the chemical compound under conditions
suitable for activation of the GALR2 receptor, and
measuring the second messenger response in the presence
and in the absence of the chemical compound, a change in
the second messenger response in the presence of the

10 chemical compound indicating that the compound activates
the GALR2 receptor.

This invention further provides a process for determining
whether a chemical compound specifically binds to and

15 activates a GALR2 receptor, which comprises contacting
a membrane fraction from a cell extract of cells
producing a second messenger response and expressing on
their cell surface the GALR2 receptor, wherein such cells
do not normally express the GALR2 receptor, with the

20 chemical compound under conditions suitable for
activation of the GALR2 receptor, and measuring the
second messenger response in the presence and in the
absence of the chemical compound, a change in the second
messenger response in the presence of the chemical

25 compound indicating that the compound activates the GALR2
receptor.

In one embodiment of the above processes, the second
messenger response comprises adenylate cyclase activity

30 and the change in second messenger response is a decrease
in adenylate cyclase activity. In one embodiment,
adenylate cyclase activity is determined by measurement
of cyclic AMP levels.

35 In another embodiment of the above processes, the second
messenger response comprises arachidonic acid release and
the change in second messenger response is an increase in

arachidonic acid levels.

In another embodiment of the above processes, the second
messenger response comprises intracellular calcium levels

5   and the change in second messenger response is an
increase in intracellular calcium levels.

In a still further embodiment of the above processes, the
second messenger response comprises inositol phospholipid

10   hydrolysis and the change in second messenger response is
an increase in inositol phospholipid hydrolysis.

This invention further provides a process for determining
whether a chemical compound specifically binds to and

15   inhibits activation of a GALR2 receptor, which comprises
separately contacting cells producing a second messenger
response and expressing on their cell surface the GALR2
receptor, wherein such cells do not normally express the
GALR2 receptor, with both the chemical compound and a

20   second chemical compound known to activate the GALR2
receptor, and with only the second compound, under
conditions suitable for activation of the GALR2 receptor,
and measuring the second messenger response in the
presence of only the second chemical compound and in the

25   presence of both the second chemical compound and the
chemical compound, a smaller change in the second
messenger response in the presence of both the chemical
compound and the second chemical compound than in the
presence of only the second chemical compound indicating

30   that the chemical compound inhibits activation of the
GALR2 receptor.

This invention further provides a process for determining
whether a chemical compound specifically binds to and

35   inhibits activation of a GALR2 receptor, which comprises
separately contacting a membrane fraction from a cell
extract of cells producing a second messenger response

and expressing on their cell surface the GALR2 receptor,
wherein such cells do not normally express the GALR2
receptor, with both the chemical compound and a second
chemical compound known to activate the GALR2 receptor,

5   and with only the second chemical compound, under
conditions suitable for activation of the GALR2 receptor,
and measuring the second messenger response in the
presence of only the second chemical compound and in the
presence of both the second chemical compound and the

10   chemical compound, a smaller change in the second
messenger response in the presence of both the chemical
compound and the second chemical compound than in the
presence of only the second chemical compound indicating
·that the chemical compound inhibits activation of the

15   GALR2 receptor.

In one embodiment of the above processes, the second
messenger response comprises adenylate cyclase activity
and the change in second messenger response is a smaller

20   decrease in the level of adenylate cyclase activity in
the presence of both the chemical compound and the second
chemical compound than in the presence of only the second
chemical compound. In one embodiment, adenylate cyclase
activity is determined by measurement of cyclic AMP

25   levels.

In another embodiment of the above processes the second
messenger response comprises arachidonic acid release,
and the change in second messenger response is a smaller

30   increase in arachidonic acid levels in the presence of
both the chemical compound and the second chemical
compound than in the presence of only the second chemical
compound.

35   In another embodiment of the above processes the second
messenger response comprises intracellular calcium
levels, and the change in second messenger response is a

smaller increase in intracellular calcium levels in the
presence of both the chemical compound and the second
chemical compound than in the presence of only the second
chemical compound.

5

In yet another embodiment of the above processes, the
second messenger response comprises inositol phospholipid
hydrolysis, and the change in second messenger response
is a smaller increase in inositol phospholipid hydrolysis
10   in the presence of both the chemical compound and the
second chemical compound than in the presence of only the
second chemical compound.

In an embodiment of any of the above processes, the GALR2
15   receptor is a mammalian GALR2 receptor. In another
embodiment of the above processes, the GALR2 receptor is
a rat GALR2 receptor or a human GALR2 receptor. In still
another embodiment of the above processes, the GALR2
receptor has the same or substantially the same amino
20   acid sequence as encoded by the plasmid K985 ATCC
Accession No. 97426), or plasmid K1045 (ATCC Accession
No. 97778). In a still further embodiment of the above
processes, the GALR2 receptor has the same or
substantially the same amino acid sequence as that shown
25   in Figure 2 (Seq. ID No. 8). In another embodiment of
the above processes, the GALR2 receptor has the same or
substantially the same amino acid sequence as the amino
acid sequence encoded by the plasmid BO29 (ATCC Accession
No. 97735) or the plasmid BO39 (ATCC Accession No.
30   _____). In a still further embodiment of the above
processes, the GALR2 receptor has the same or
substantially the same amino acid sequence as that shown
in Figure 11 (Seq. ID No. 30).

35   In an embodiment of any of the above processes, the cell
is an insect cell. In another embodiment of any of the
above processes, the cell is a mammalian cell. In still

- 51 -

further embodiments, the cell is nonneuronal in origin.

In another embodiment of the above processes, the
nonneuronal cell is a COS-7 cell, 293 human embryonic
5   kidney cell, NIH-3T3 cell, a mouse Y1 cell or LM(tk-)
cell. In still further embodiments, nonneuronal cell is
the LM(tk-) cell designated L-rGALR2-8 (ATCC Accession
No. CRL-12074), the LM(tk-) cell L-rGALR2I-4 (ATCC
Accession No. CRL-12223, or the CHO cell C-rGalR2-79
10   (ATCC Accession No. _____).

This invention further provides a compound determined by
any of the above processes. In another embodiment, the
compound is not previously known to bind to a GALR2
15   receptor.

This invention provides a method of screening a plurality
of chemical compounds not known to bind to a GALR2
receptor to identify a compound which specifically binds
20   to the GALR2 receptor, which comprises (a) contacting
cells transfected with and expressing DNA encoding the
GALR2 receptor with a compound known to bind specifically
to the GALR2 receptor; (b) contacting the preparation of
step (a) with the plurality of compounds not known to
25   bind specifically to the GALR2 receptor, under conditions
permitting binding of compounds known to bind the GALR2
receptor; (c) determining whether the binding of the
compound known to bind to the GALR2 receptor is reduced
in the presence of the compounds, relative to the binding
30   of the compound in the absence of the plurality of
compounds; and if so (d) separately determining the
binding to the GALR2 receptor of each compound included
in the plurality of compounds, so as to thereby identify
the compound which specifically binds to the GALR2
35   receptor.

This invention provides a method of screening a plurality

of chemical compounds not known to bind to a GALR2
receptor to identify a compound which specifically binds
to the GALR2 receptor, which comprises (a) preparing a
cell extract from cells transfected with and expressing
5   DNA encoding the GALR2 receptor, isolating a membrane
fraction from the cell extract, contacting the membrane
fraction with a compound known to bind specifically to
the GALR2 receptor; (b) contacting the preparation of
step (a) with the plurality of compounds not known to
10   bind specifically to the GALR2 receptor, under conditions
permitting binding of compounds known to bind the GALR2
receptor; (c) determining whether the binding of the
compound known to bind to the GALR2 receptor is reduced
in the presence of the compounds, relative to the binding
15   of the compound in the absence of the plurality of
compounds; and if so (d) separately determining the
binding to the GALR2 receptor of each compound included
in the plurality of compounds, so as to thereby identify
the compound which specifically binds to the GALR2
20   receptor.

In one embodiment of the above-described methods, the
GALR2 receptor is a rat GALR2 receptor. In another
embodiment, the GALR2 receptor has the same or
25   substantially the same amino acid sequence as the amino
acid sequence shown in Figure 2 (Seq. I.D. No. 8). In
yet another embodiment, the GALR2 receptor has the amino
acid sequence shown in Figure 2 (Seq. I.D. No. 8). In
another embodiment, the GALR2 receptor is a human GALR2
30   receptor. In still another embodiment, the GALR2
receptor has the same or substantially the same amino
acid sequence as the amino acid sequence encoded by
plasmid BO29 or plasmid BO39. In another embodiment, the
GALR2 receptor has the same or substantially the same
35   amino acid sequence as the amino acid sequence shown in
Figure 11 (Seq. I.D. No. 30). In yet another embodiment,
the GALR2 receptor has the amino acid sequence shown in

Figure 11 (Seq. I.D. No. 30).

This invention provides a method of screening a plurality
of chemical compounds not known to activate a GALR2
5   receptor to identify a compound which activates the GALR2
receptor which comprises (a) contacting cells transfected
with and expressing the GALR2 receptor with the plurality
of compounds not known to activate the GALR2 receptor,
under conditions permitting activation of the GALR2
10   receptor; (b) determining whether the activity of the
GALR2 receptor is increased in the presence of the
compounds; and if so (c) separately determining whether
the activation of the GALR2 receptor is increased by each
compound included in the plurality of compounds, so as to
15   thereby identify the compound which activates the GALR2
receptor.

This invention provides a method of screening a plurality
of chemical compounds not known to activate a GALR2
20   receptor to identify a compound which activates the GALR2
receptor which comprises (a) preparing a cell extract
from cells transfected with and expressing DNA encoding
the GALR2 receptor, isolating a membrane fraction from
the cell extract, contacting the membrane fraction with
25   the plurality of compounds not known to activate the
GALR2 receptor, under conditions permitting activation of
the GALR2 receptor; (b) determining whether the activity
of the GALR2 receptor is increased in the presence of the
compounds; and if so (c) separately determining whether
30   the activation of the GALR2 receptor is increased by each
compound included in the plurality of compounds, so as to
thereby identify the compound which activates the GALR2
receptor.

35   In an embodiment of the above-described methods, the
GALR2 receptor is a rat GALR2 receptor. In still another
embodiment, the GALR2 receptor has the same or

- 54 -

substantially the same amino acid sequence as the amino acid sequence shown in Figure 2 (Seq. I.D. No.8). In yet another embodiment, the GALR2 receptor has the amino acid sequence shown in Figure 2 (Seq. I.D. No. 8). In another

5   embodiment, the GALR2 receptor is a human GALR2 receptor. In still another embodiment, the GALR2 receptor has the same or substantially the same amino acid sequence as the amino acid sequence encoded by plasmid BO29 or plasmid BO39. In another embodiment, the GALR2 receptor has the

10   same or substantially the same amino acid sequence as the amino acid sequence shown in Figure 11 (Seq. I.D. No. 30). In yet another embodiment, the GALR2 receptor has the amino acid sequence shown in Figure 11 (Seq. I.D. No. 30).

15

This invention provides a method of screening a plurality of chemical compounds not known to inhibit the activation of a GALR2 receptor to identify a compound which inhibits the activation of the GALR2 receptor, which comprises (a)

20   contacting cells transfected with and expressing the GALR2 receptor with the plurality of compounds in the presence of a known GALR2 receptor agonist, under conditions permitting activation of the GALR2 receptor; (b) determining whether the activation of the GALR2

25   receptor is reduced in the presence of the plurality of compounds, relative to the activation of the GALR2 receptor in the absence of the plurality of compounds; and if so (c) separately determining the inhibition of activation of the GALR2 receptor for each compound

30   included in the plurality of compounds, so as to thereby identify the compound which inhibits the activation of the GALR2 receptor.

This invention provides a method of screening a plurality of chemical compounds not known to inhibit the activation

35   of a GALR2 receptor to identify a compound which inhibits the activation of the GALR2 receptor, which comprises (a)

preparing a cell extract from cells transfected with and
expressing DNA encoding the GALR2 receptor, isolating a
membrane fraction from the cell extract, contacting the
membrane fraction with the plurality of compounds in the

5   presence of a known GALR2 receptor agonist, under
conditions permitting activation of the GALR2 receptor;
(b) determining whether the activation of the GALR2
receptor is reduced in the presence of the plurality of
compounds, relative to the activation of the GALR2

10   receptor in the absence of the plurality of compounds;
and if so (c) separately determining the inhibition of
activation of the GALR2 receptor for each compound
included in the plurality of compounds, so as to thereby
identify the compound which inhibits the activation of

15   the GALR2 receptor.

In an embodiment of the above-described methods, the
GALR2 receptor is a rat GALR2 receptor. In another
embodiment, the GALR2 receptor has the same or

20   substantially the same amino acid sequence as the amino
acid sequence shown in Figure 2 (Seq. I.D. No.8). In yet
another embodiment, the GALR2 receptor has the amino acid
sequence shown in Figure 2 (Seq. I.D. No. 8). In another
embodiment, the GALR2 receptor is a human GALR2 receptor.

25   In still another embodiment, the GALR2 receptor has the
same or substantially the same amino acid sequence as the
amino acid sequence encoded by plasmid BO29 or plasmid
BO39. In another embodiment, the GALR2 receptor has the
same or substantially the same amino acid sequence as the

30   amino acid sequence shown in Figure 11 (Seq. I.D. No.
30). In yet another embodiment, the GALR2 receptor has
the amino acid sequence shown in Figure 11 (Seq. I.D. No.
30).

35   In one embodiment of any of the above-described methods,
the activation of the GALR2 receptor is determined by a
second messenger assay. In an embodiment, the second

- 56 -

messenger assay measures adenylate cyclase activity. In other embodiments, the second messenger is cyclic AMP, intracellular calcium, or arachidonic acid or a phosphoinositol lipid metabolite. Second messenger

5   coupling may also be measured by assaying the binding of GTP gamma S to membranes.

This invention further provides a method of measuring GALR2 receptor activation in an oocyte expression system

10   such as a Xenopus oocyte or melanophore. In an embodiment, receptor activation is determined by measurement of ion channel activity.

Expression of genes in Xenopus oocytes is well known in

15   the art (A. Coleman, _Transcription and Translation: A Practical Approach_ (B.D. Hanes, S.J. Higgins, eds., pp 271-302, IRL Press, Oxford, 1984; Y. Masu et al., _Nature_ 329:21583-21586, 1994) and is performed using microinjection of native mRNA or _in vitro_ synthesized

20   mRNA into frog oocytes. The preparation of _in vitro_ synthesized mRNA can be performed by various standard techniques (J. Sambrook et al., _Molecular Cloning: A Laboratory Manual_, Second Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989) including

25   using T7 polymerase with the mCAP RNA capping kit (Stratagene).

In a further embodiment of the invention, the cell is a mammalian cell. In another embodiment of the invention,

30   the mammalian cell is non-neuronal in origin. In still further embodiments of the invention, the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a mouse Y1 cell, a LM(tk-) cell, a CHO cell, or an NIH-3T3 cell. In an embodiment of the invention, the

35   nonneuronal cell is the LM(tk-) cell designated L-rGALR2-8 (ATCC Accession No. CRL-12074), the LM(tk-) cell L-rGALR2I-4 (ATCC Accession No. CRL-12223, or the CHO cell

C-rGalR2-79 (ATCC Accession No. _____).

This invention provides a pharmaceutical composition
comprising a compound identified by the above-described
5   methods and a pharmaceutically acceptable carrier.

In an embodiment of the above-described methods, the cell
is non-neuronal in origin. In a further embodiment, the
non-neuronal cell is a COS-7 cell, 293 human embryonic
10   kidney cell, NIH-3T3 cell, a mouse Y1 cell or LM(tk-)
cell.

In one embodiment of the above-described methods, the
compound is not previously known.
15

This invention provides a GALR2 receptor agonist detected
by the above-described methods. This invention provides
a GALR2 receptor antagonist detected by the above-
described methods. In an embodiment the cell is a non-
20   mammalian cell, for example, a Xenopus oocyte or
melanophore. In another embodiment the cell is a
neuronal cell, for example, a glial cell line such as C6.
In an embodiment, the cell is non-neuronal in origin. In
a further embodiment, the cell is a Cos-7 or a CHO cell,
25   a 293 human embryonic kidney cell, an LM(tk-) cell or an
NIH-3T3 cell. In an embodiment of the invention, the
LM(tk-) cell is the cell designated L-rGALR2-8 (ATCC
Accession No. CRL-12074), the LM(tk-) cell L-rGALR2I-4
(ATCC Accession No. CRL-12223, or the CHO cell C-rGalR2-
30   79 (ATCC Accession No. _____).

This invention provides a pharmaceutical composition
comprising a drug candidate identified by the above-
described methods and a pharmaceutically acceptable
35   carrier.

This invention provides a method for determining whether

a chemical compound is a GALR2 antagonist which comprises: (a) administering to an animal a GALR2 agonist and measuring the amount of food intake in the animal; (b) administering to a second animal both the GALR2 agonist and the chemical compound, and measuring the amount of food intake in the second animal; and (c) determining whether the amount of food intake is reduced in the presence of the chemical compound relative to the amount of food intake in the absence of the compound, so as to thereby determine whether the compound is a GALR2 antagonist. This invention further provides a method of screening a plurality of chemical compounds to identify a chemical compound which is a GALR2 antagonist which comprises: (a) administering to an animal a GALR2 agonist and measuring the amount of food intake in the animal; (b) administering to a second animal the GALR2 agonist and at least one chemical compound of the plurality of compounds, and measuring the amount of food intake in the animal; (c) determining whether the amount of food intake is reduced in the presence of at least one chemical compound of the plurality of chemical compounds relative to the amount of food intake in the absence of at least one of the compounds, and if so; (d) separately determining whether each chemical compound is a GALR2 antagonist according to the method described above, so as to thereby determine if the chemical compound is a GALR2 antagonist. In one embodiment the GALR2 agonist is [D-Trp]$_2$-galanin $_{(1-29)}$. In another embodiment the animal is a non-human mammal. In a further embodiment, the animal is a rodent.

This invention provides a method of detecting expression of a GALR2 receptor by detecting the presence of mRNA coding for the GALR2 receptor which comprises obtaining total mRNA from a cell or tissue sample and contacting the mRNA so obtained with the above-described nucleic acid probe under hybridizing conditions, detecting the

presence of mRNA hybridized to the probe, and thereby detecting the expression of the GALR2 receptor by the cell or in the tissue.

This invention provides a method of treating an abnormality in a subject, wherein the abnormality is alleviated by administering to the subject an amount of a GALR2 selective compound, effective to treat the abnormality. Abnormalities which may be treated include cognitive disorder, pain, sensory disorder (olfactory, visual), motor coordination abnormality, motion sickness, neuroendocrine disorders, sleep disorders, migraine, Parkinson's disease, hypertension, heart failure, convulsion/epilepsy, traumatic brain injury, diabetes, glaucoma, electrolyte imbalances, respiratory disorders (asthma, emphysema), depression, reproductive disorders, gastric and intestinal ulcers, gastroesophageal reflux disorder, gastric hypersecretion, gastrointestinal motility disorders (diarrhea), inflammation, immune disorders, and anxiety. In one embodiment the compound is an agonist. In another embodiment the compound is an antagonist.

This invention provides a method of treating an abnormality in a subject, wherein the abnormality is alleviated by the inhibition of a GALR2 receptor which comprises administering to a subject an effective amount of the above-described pharmaceutical composition effective to decrease the activity of the GALR2 receptor in the subject, thereby treating the abnormality in the subject. In an embodiment, the abnormality is obesity. In another embodiment, the abnormality is bulimia.

This invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by the activation of a GALR2 receptor which comprises administering to a subject an effective amount

- 60 -

of the above-described pharmaceutical composition
effective to activate the GALR2 receptor in the subject.
In an embodiment, the abnormal condition is anorexia.

5   In another embodiment, the compound binds selectively to
a GALR2 receptor. In yet another embodiment, the
compound binds to the GALR2 receptor with an affinity
greater than ten-fold higher than the affinity with which
the compound binds to a GALR1 receptor. In a still
10   further embodiment, the compound binds to the GALR2
receptor with an affinity greater than ten-fold higher
than the affinity with which the compound binds to a
GALR3 receptor.

15   This invention provides a method of detecting the
presence of a GALR2 receptor on the surface of a cell
which comprises contacting the cell with the above-
described antibody under conditions permitting binding of
the antibody to the receptor, detecting the presence of
20   the antibody bound to the cell, and thereby detecting the
presence of a GALR2 receptor on the surface of the cell.

This invention provides a method of determining the
physiological effects of varying levels of activity of
25   GALR2 receptors which comprises producing a transgenic
nonhuman mammal whose levels of GALR2 receptor activity
are varied by use of an inducible promoter which
regulates GALR2 receptor expression.

30   This invention provides a method of determining the
physiological effects of varying levels of activity of
GALR2 receptors which comprises producing a panel of
transgenic nonhuman mammals each expressing a different
amount of GALR2 receptor.

35

This invention provides a method for identifying an
antagonist capable of alleviating an abnormality wherein

the abnormality is alleviated by decreasing the activity
of a GALR2 receptor comprising administering a compound
to the above-described transgenic nonhuman mammal and
determining whether the compound alleviates the physical

5   and behavioral abnormalities displayed by the transgenic
nonhuman mammal as a result of overactivity of a GALR2
receptor, the alleviation of the abnormality identifying
the compound as an antagonist.

10   This invention provides an antagonist identified by the
above-described methods.   This invention provides a
pharmaceutical composition comprising an antagonist
identified by the above-described methods and a
pharmaceutically acceptable carrier.

15

This invention provides a method of treating an
abnormality in a subject wherein the abnormality is
alleviated by decreasing the activity of a GALR2 receptor
which comprises administering to a subject an effective

20   amount of the above-described pharmaceutical composition,
thereby treating the abnormality.

This invention provides a method for identifying an
agonist capable of alleviating an abnormality in a

25   subject wherein the abnormality is alleviated by
increasing the activity of a GALR2 receptor comprising
administering a compound to a transgenic nonhuman mammal
and determining whether the compound alleviates the
physical and behavioral abnormalities displayed by the

30   transgenic nonhuman mammal, the alleviation of the
abnormality identifying the compound as an agonist.

This invention provides an agonist identified by the
above-described methods.

35

This invention provides a pharmaceutical composition
comprising an agonist identified by the above-described

- 62 -

methods and a pharmaceutically acceptable carrier.

This invention provides a method for treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a GALR2 receptor which comprises administering to a subject an effective amount of the above-described pharmaceutical composition, thereby treating the abnormality.

This invention provides a method for diagnosing a predisposition to a disorder associated with the activity of a specific human GALR2 receptor allele which comprises: (a) obtaining DNA of subjects suffering from the disorder; (b) performing a restriction digest of the DNA with a panel of restriction enzymes; (c) electrophoretically separating the resulting DNA fragments on a sizing gel; (d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human GALR2 receptor and labelled with a detectable marker; (e) detecting labelled bands which have hybridized to DNA encoding a human GALR2 receptor labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; (f) preparing DNA obtained for diagnosis by steps a-e; and (g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

In an embodiment, a disorder associated with the activity of a specific human GALR2 receptor allele is diagnosed. In another embodiment, the above-described method may be used to identify a population of patients having a

specific GALR2 receptor allele, in which population the
disorder may be alleviated by administering to the
subjects a GALR2-selective compound.

5   This invention provides a method of preparing the
purified GALR2 receptor which comprises: (a) inducing
cells to express GALR2 receptor; (b) recovering the
receptor from the induced cells; and (c) purifying the
receptor so recovered.

10

This invention provides a method of preparing a purified
GALR2 receptor which comprises: (a) inserting nucleic
acid encoding the GALR2 receptor in a suitable vector;
(b) introducing the resulting vector in a suitable host

15   cell; (c) placing the resulting cell in suitable
condition permitting the production of the isolated GALR2
receptor; (d) recovering the receptor produced by the
resulting cell; and (e) purifying the receptor so
recovered.

20

This invention provides a method of modifying feeding
behavior of a subject which comprises administering to
the subject an amount of a compound which is a galanin
receptor agonist or antagonist effective to increase or

25   decrease the consumption of food by the subject so as to
thereby modify feeding behavior of the subject. In one
embodiment, the compound is a GALR2 receptor antagonist
and the amount is effective to decrease the consumption
of food by the subject. In another embodiment the

30   compound is administered in combination with food.

In yet another embodiment the compound is a GALR2
receptor agonist and the amount is effective to increase
the consumption of food by the subject. In a still

35   further embodiment, the compound is administered in
combination with food. In other embodiments the subject
is a vertebrate, a mammal, a human or a canine.

- 64 -

In one embodiment, the compound binds selectively to a GALR2 receptor. In another embodiment, the compound binds to the GALR2 receptor with an affinity greater than ten-fold higher than the affinity with which the compound

5   binds to a GALR1 receptor. In another embodiment, the compound binds to the GALR2 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to a GALR3 receptor. In yet another embodiment, the compound binds to the GALR2 receptor with

10   an affinity greater than one hundred-fold higher than the affinity with which the compound binds to a GALR1 receptor. In another embodiment, the compound binds to the GALR2 receptor with an affinity greater than one hundred-fold higher than the affinity with which the

15   compound binds to a GALR3 receptor.

This invention provides a method of treating Alzheimer's disease in a subject which comprises administering to the subject an amount of a compound which is a galanin

20   receptor antagonist effective to treat the subject's Alzheimer's disease. In one embodiment, the galanin receptor antagonist is a GALR2 receptor antagonist and the amount of the compound is effective to treat the subject's Alzheimer's disease.

25

This invention provides a method of producing analgesia in a subject which comprises administering to the subject an amount of a compound which is a galanin receptor agonist effective to produce analgesia in the subject.

30   In another embodiment, the galanin receptor agonist is a GALR2 receptor agonist and the amount of the compound is effective to produce analgesia in the subject.

This invention provides a method of decreasing

35   nociception in a subject which comprises administering to the subject an amount of a compound which is a GALR2 receptor agonist effective to decrease nociception in the

subject.

This invention provides a method of treating pain in a
subject which comprises administering to the subject an
amount of a compound which is a GALR2 receptor agonist
effective to treat pain in the subject.

This invention provides a method of decreasing feeding
behavior of a subject which comprises administering a
compound which is a GALR2 receptor antagonist and a
compound which is a Y5 receptor antagonist, the amount of
such antagonists being effective to decrease the feeding
behavior of the subject. In one embodiment, the GALR2
antagonist and the Y5 antagonist are administered in
combination. In another embodiment, the GALR2 antagonist
and the Y5 antagonist are administered once. In another
embodiment, the GALR2 antagonist and the Y5 antagonist
are administered separately. In still another
embodiment, the GALR2 antagonist and the Y5 antagonist
are administered once. In another embodiment, the
galanin receptor antagonist is administered for about 1
week to 2 weeks. In another embodiment, the Y5 receptor
antagonist is administered for about 1 week to 2 weeks.

In yet another embodiment, the GALR2 antagonist and the
Y5 antagonist are administered alternately. In another
embodiment, the GALR2 antagonist and the Y5 antagonist
are administered repeatedly. In a still further
embodiment, the galanin receptor antagonist is
administered for about 1 week to 2 weeks. In another
embodiment, the Y5 receptor antagonist is administered
for about 1 week to 2 weeks.

This invention also provides a method as described above,
wherein the compound is administered in a pharmaceutical
composition comprising a sustained release formulation.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

- 67 -

Experimental Details

Materials and Methods

<u>Construction and screening of a rat hypothalamus cDNA</u>
<u>library</u>

Total RNA was prepared from rat hypothalami by a
modification of the guanidine thiocyanate method
(Chirgwin, 1979). Poly A⁺ RNA was purified using a
FastTrack kit (Invitrogen Corp., San Diego, CA). Double
stranded (ds) cDNA was synthesized from 4.6 μg of poly A⁺
RNA according to Gubler and Hoffman (1983) with minor
modifications. The resulting cDNA was ligated to
BstXI/EcoRI adaptors (Invitrogen Corp.) and the excess
adaptors removed by exclusion column chromatography.
High molecular weight fractions of size-selected ds-cDNA
were ligated in pEXJ.T7 (an Okayama and Berg expression
vector modified from pcEXV (Miller & Germain, 1986) to
contain BstXI and other additional restriction sites and
a T7 promoter (Stratagene) and electroporated in E.coli
MC 1061 (Gene Pulser, Biorad). A total of 3×10^6
independent clones with a mean insert size of 2.2 kb were
generated. The library was plated on agar plates
(Ampicillin selection) in 584 primary pools of ~5,000
independent clones. After 18 hours amplification, the
bacteria from each pool were scraped, resuspended in 4 mL
of LB media and 0.75 mL processed for plasmid
purification (QIAwell-96 ultra, Qiagen,Inc., Chatsworth,
CA). Aliquots of each bacterial pool were stored at -85°C
in 20% glycerol.

To screen the library, COS-7 cells were plated in slide
chambers (Lab-Tek) in Dulbecco's modified Eagle medium
(DMEM) supplemented with 10% calf serum, 100 U/mL of
penicillin, 100 ug/mL streptomycin, 2 mM L-glutamine
(DMEM-C) and grown at 37°C. in a humidified 5% CO_2
atmosphere for 24 hours before transfection. Cells were

- 68 -

transfected with miniprep DNA prepared from the primary
pools (~4,500 cfu/pool) of the rat hypothalamus cDNA
library using a modification of the DEAE-dextran method
(Warden & Thorne, 1968). Pools containing GALR1 were
5   identified by PCR prior to screening and were omitted
from the primary screen. The galanin binding assay was
carried out after 48 hours. Cells were rinsed twice with
phosphate-buffered saline (PBS) then incubated with 1 nM
^{125}I-porcine galanin (NEN; specific activity ~2200 Ci/mmol)
10   in 20mM HEPES-NaOH, pH 7.4, containing 1.26 mM $CaCl_2$, 0.81
mM $MgSO_4$, 0.44 mM KH_2PO_4, 5.4 mM KCl, 10 mM NaCl, 0.1% BSA,
and 0.1% bacitracin for one hour at room temperature.
After rinsing and fixation in 2.5% glutaraldehyde, slides
were rinsed in PBS, air-dried, and dipped in
15   photoemulsion (Kodak, NTB-2). After a 3-4 day exposure
slides were developed in Kodak D19 developer, fixed, and
coverslipped (Aqua-Mount, Lerner Laboratories), then
inspected for positive cells by brightfield microscopy
(Leitz Laborlux, 25X magnification). One pool with
20   positive cells, (J126) was subdivided and rescreened
repeatedly until a single colony was isolated that
conferred galanin binding. The 3.8 kb cDNA is preferably
sequenced on both strands using Sequenase (US
Biochemical, Cleveland, OH) according to the
25   manufacturer. Nucleotide and peptide sequence analyses
are performed using the Wisconsin Package (GCG, Genetics
Computer group, Madison, WI) or PC/GENE (Intelligenetics,
Mountain View, CA).

30   <u>PCR Methodology</u>
PCR reactions were carried out in 20μl volumes using Taq
Polymerase (Boehringer Mannheim, Indianapolis, IN) in a
buffer containing 10mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5
mM $MgCl_2$ 0.01% gelatin, 0.2 mM each dNTP, and 1 μM each
35   PCR primer. To prescreen library pools for GALR1, two
GALR1 primer sets were used (KS-1177/1178 and KS-
1311/1313, see below) to determine whether GALR1 was

present in original bacterial stocks of each library
pool. PCR was carried out for 40 cycles of 94°C/2 min,
68°C/2 min, 72°C/3 min. Pools positive for GALR1 by PCR
were eliminated from the library screen.

To confirm that the purified cDNA conferring galanin
binding was distinct from GALR1, the isolated clone
representing pool J126-10-334 (K985) was subjected to PCR
analysis using three GALR1 primer sets representing
different regions of GALR1. The nucleotide sequences of
the primer sets are shown below:

KS-1177: 5'-TGG GCA ACA GCC TAG TGA TCA CCG -3' (Seq.
I.D. No. 1) Nucleotides 146-169 of human GALR1 coding
region, forward primer.

KS-1178: 5'-CTG CTC CCA GCA GAA GGT CTG GTT -3' (Seq.
I.D. No. 2) Nucleotides 547-570 of human GALR1 coding
region, reverse primer.

KS-1311: 5'-CCT CAG TGA AGG GAA TGG GAG CGA -3'(Seq.
I.D. No. 3) Nucleotides 21-44 of rat GALR1 coding region,
forward primer.

KS-1313: 5'-CTC ATT GCA AAC ACG GCA CTT GAA CA -3'(Seq.
I.D. No. 4) Nucleotides 944-969 of rat GALR1 coding
region, reverse primer.

KS-1447: 5'-CTT GCT TGT ACG CCT TCC GGA AGT -3'(Seq.
I.D. No. 5) Nucleotides 920-943 of rat GALR1 coding
region, reverse primer.

KS-1448: 5'-GAG AAC TTC ATC ACG CTG GTG GTG -3'(Seq.
I.D. No. 6). Nucleotides 91-114 of rat GALR1 coding
region, forward primer.

Generation of Human GALR2 PCR product

Human genomic DNA (1 μg; 12 different lots from Promega and Clontech) were amplified in 50μl PCR reaction mixtures using the Expand Long Template PCR System (as supplied and described by the manufacturer, Boehringer

5   Mannheim) and 1μM of primers, using a program consisting of 40 cycles of 94°C for 2 min, 60°C for 2 min, and 68°C for 3 min, with a pre- and post-incubation of 95°C for 5 min and 68°C for 10 min, respectively. PCR primers for hGALR2 were designed against rGALR2 sequence: forward

10   primer NS525 in the fourth transmembrane domain, and reverse primer NS526 in the sixth transmembrane domain. The PCR products were run on a 0.8% low-melting agarose gel. The single ≈300 bp fragment from 3 different lots were isolated, purified by phenol extraction and

15   subjected to sequencing using the T7 Sequenase PCR product sequencing kit (Amersham). Sequence was analyzed using the Wisconsin Package (GCG, Genetics Computer Group, Madison, WI).

20   <u>5' and 3' RACE Analysis of Human GALR2</u>
     5' and 3' RACE (rapid analysis of cDNA ends) were performed on human brain and human lung RNAs (Clontech), respectively, using a Marathon cDNA Amplification Kit (Clontech). Total RNA was poly A+

25   selected using a FastTrack mRNA Isolation Kit (Invitrogen Corp., San Diego, CA). For 5' RACE, double stranded (ds) cDNA was synthesized from 1 μg Poly A+ RNA using BB 153, a reverse primer from the 5' end of the sixth transmembrane domain of hGALR2 from the PCR fragment

30   described above. Adaptor ligation and nested PCR were performed according to the Marathon cDNA Amplification protocol using Advantage KlenTaq Polymerase (Clontech). The initial PCR reaction was performed on 1 μl of a 50 fold dilution of the ligated cDNA using the supplier's

35   Adaptor Primer 1 and BB 154, a reverse primer from the fifth transmembrane domain of the hGALR2 PCR product above. One μl of this initial PCR reaction was re-

amplified using the Adaptor Primer 2 and NS 563, a
reverse primer just upstream from BB154. The conditions
for PCR were 30 sec at 94°C, 4 min at 72°C for 5 cycles,
30 sec at 94°C, 4 min at 70°C for 5 cycles, 20 sec at
5   94°C, 4 min at 68°C for 25 cycles, with a pre- and post-
incubation of 1 min at 94°C and 7 min at 68°C
respectively. A 600 base pair fragment from the nested
PCR was isolated from a 1% TAE gel using a GENECLEAN III
kit (BIO 101, Vista, CA) and sequenced using AmpliTaq DNA
10   Polymerase, FS (Perkin Elmer). The sequence was run on
an ABI PRISM 377 DNA Sequencer and analyzed using the
Wisconsin Package (GCG, Genetics Computer Group, Madison,
WI). For 3' RACE, double stranded (ds) cDNA was
synthesized from 1 μg Poly A+ RNA using the cDNA
15   synthesis primer CDS supplied with the Marathon cDNA
Amplification Kit (Clontech). PCR conditions for 3' RACE
were similar to 5' RACE except that BB166 and BB167,
forward primers from the fifth transmembrane domain of
the hGALR2 PCR fragment described above, were used in
20   place of BB154 and NS563, respectively. A 500 base pair
fragment from the nested PCR was isolated from a 1% TAE
gel using a GENECLEAN III kit (BIO 101, Vista, CA) and
sequenced as above.

25   Construction and Screening of a Human Heart cDNA Library
Poly A+ RNA was purified from human heart RNA (Clontech)
using a FastTrack kit (Invitrogen, Corp.). DS- cDNA was
synthesized from 8 μg of poly A+ RNA according to Gubler
and Hoffman (1983) with minor modifications. The
30   resulting cDNA was ligated to BstXI adaptors (Invitrogen,
Corp.) and the excess adaptors removed by exclusion
column chromatography. High molecular weight fractions
of size-selected ds-cDNA were ligated in pEXJ.BS, an
Okayama and Berg expression vector modified from pcEXV
35   (Miller and Germain, 1986) to contain BstXI and other
additional restriction sites. A total of 4.45X 10^6
independent clones with a mean insert size of 2.5 kb were

- 72 -

generated.   The library was plated on agar plates
(Ampicillin selection) in 127 primary pools; 50 pools
with 37,500 independent clones, 51 pools with 25,000
clones and 26 pools with 50,000 clones. Glycerol stocks
5   of the primary pools were combined in 16 superpools of 8
and screened for hGlR2 by PCR using primers BB153 and
BB169, a forward primer from the second intracellular
domain of hGALR2 identified in the 5' RACE fragment
above. PCR was performed with the Expand Long Template
10   PCR System (Boehringer Mannheim) under the following
conditions: 1 min at 94°C, 4 min at 68°C for 40 cycles,
with a pre- and post-incubation of 5 min at 95°C and 7
min at 68°C, respectively. Primary pools from positive
superpools were screened by PCR and then primary pool 169
15   was subdivided and screened by PCR.   One positive
subpool, 69-11, was subdivided into 20 pools of 1200
clones plated on agar plates (ampicillin selection).
Colonies were transferred to nitrocellulose membranes
(Schleicher and Schuell, Keene, NH), denatured in 0.4 N
20   NaOH, 1.5 M NaCl, renatured in 1M Tris, 1.5 M NaCl, and
UV cross-linked.   Filters were hybridized overnight at
40°C in a buffer containing 50 % formamide, 5X SSC, 7 mM
TRIS, 1X Denhardt's solution and 25 μg/ml salmon sperm DNA
(Sigma Chemical Co.) and 10^6 cpm/ml of KS1567, an
25   oligonucleotide probe from the 3' end of the fifth
transmembrane domain of hGALR2, labeled with γ-^{32}P[ATP]
(6000Ci/mmol, NEN) using polynucleotide kinase
(Boehringer Mannheim).   Filters were washed 2 x 15
minutes at room temperature in 2XSSC, 0.1% SDS, 2 x 15
30   minutes at 50°C in 0.1XSSC, 0.1% SDS, and exposed to XAR
X-ray film (Kodak) for 3 days. Colonies which appeared
to hybridize were re-screened by PCR using primers BB167
and BB170, a reverse primer from the COOH terminus of
hGlR2 identified by the 3' RACE fragment above. PCR was
35   performed with the Expand Long Template PCR System
(Boehringer Mannheim) under the following conditions: 1
min at 94°C, 2 min at 58°C, 2 min at 68°C for 28 cycles,

- 73 -

with a pre- and post-incubation of 5 min at 95°C and 7
min at 68°C respectively. One positive colony, 69-11-5
was amplified overnight in 10 ml LB media and processed
for plasmid purification using a standard alkaline lysis
5   miniprep procedure followed by a PEG precipitation. To
ensure that 69-11-5 was a single colony, it was amplified
for 3 hours in 3 ml of LB media and then 1 μl of a 1:100
dilution was plated on an agar plate. Twenty colonies
were screened by PCR using primers BB167 and BB170 using
10   the same conditions as above, except that 25 cycles were
used instead of 28. One positive single colony, 69-11-5-
3, designated BO29, was amplified overnight in 10 ml of
TB media and processed for plasmid purification. Vector-
anchored PCR was performed on BO29 using the Expand Long
15   Template PCR System (Boehringer Mannheim) to determine
the orientation and size of the insert. BB173 and BB172,
forward and reverse vector primers, respectively, were
used with primers BB169 and BB153. The conditions for
PCR were 1 min at 94°C, 4 min at 68°C for 36 cycles, with
20   a pre- and post-incubation of 5 min at 95°C and 7 min at
68°C respectively. BO29 is preferably sequenced on both
strands using AmpliTaq DNA Polymerase, FS (Perkin Elmer).
The sequence is run on an ABI PRISM 377 DNA Sequencer and
analyzed using the Wisconsin Package (GCG, Genetics
25   Computer Group, Madison, WI).

To test the ability of 69-11-5 to confer galanin binding,
COS-7 cells were plated in slide chambers (Lab-Tek) in
Dulbecco's modified Eagle medium (DMEM) supplemented with
30   10% calf serum, 100U/ml of penicillin, 100 μg/ml
streptomycin, 2mM L-glutamine (DMEM-c) and grown at 37°C
in a humidified 5% CO_2 atmosphere for 24 hours before
transfection. Cells were transfected with 1 μg of
miniprep DNA from 69-11-5 or vector control using a
35   modification of the DEAE-dextran method (Warden and
Thorne, 1968). 48 hours after transfection, cells were
rinsed with phosphate-buffered saline (PBS) then

incubated with 1 nM ^{125}I-rat galanin (NEN; specific
activity ~2200 Ci/mmol) and 2 nM ^{125}I-porcine galanin (NEN;
specific activity ~2200 Ci/mmol) in 20mM HEPES-NaOH, pH
7.4, containing 1.26 mM $CaCl_2$, 0.81 mM $MgSO_4$, 0.44 mM

5   KH_2PO_4, 5.4 mM KCl, 10 mM NaCl, 0.1% BSA, and 0.1%
bacitracin for one hour at room temperature. After
rinsing and fixation in 2.5% glutaraldehyde, slides were
rinsed in PBS, air-dried, and dipped in photoemulsion
(Kodak, NTB-2). After a 4-day exposure, slides were

10   developed in Kodak D19 developer, fixed, and coverslipped
(Aqua-Mount, Lerner Laboratories), then inspected for
positive cells by brightfield microscopy (Leitz Laborlux,
25X magnification). To test the ability of the single
clone B029 to confer galanin binding, B029 or control

15   vector were transfected into COS-7 cells for testing of
^{125}I galanin as described above, with the exception that
after fixation, binding of ^{125}I galanin to cells on the
slide was detected using an ^{125}I probe (Mini-Instruments,
Ltd., Essex, England). The signal from B029 transfected

20   cells was compared with the signal from control vector
transfected cells.

Primers and probes used

25   NS525: 5'CCCTACCTGAGCTACTACCGTCA 3' (SEQ ID NO:15);

NS526: 5'ACCAAACCACACGCAGAGGATAAG 3'(SEQ ID NO:16);

BB153: 5'-CCACGATGAGGATCATGCGTGTCACC-3'(SEQ ID NO:17);

30

BB154: 5'-TAGGTCAGGCCGAGAACCAGCACAGG-3' (SEQ ID NO:18);

NS563: 5'-CAGGTAGCTGAAGACGAAGGTGCA-3'(SEQ ID NO:19);

35   BB166: 5'-CTGCACCTTCGTCTTCAGCTACCTG-3'(SEQ ID NO:20);

BB167: 5'-CCTGTGCTGGTTCTCGGCCTGACCTA-3'(SEQ ID NO:21);

BB169: 5'-TATCTGGCCATCCGCTACCCGCTGCA-3'(SEQ ID NO:22);

KS1567: 5'-TTGCGCTACCTCTGGCGCGCCGTCGACCCGGTGGCCGCGGGCTCG-3' (SEQ ID NO:23);

BB170: 5'-CCAACAATGACTCCAACTCTGTGAC-3'(SEQ ID NO:24);

BB173: 5'-AGGCGCAGAACTGGTAGGTATGGAA-3'(SEQ ID NO:25);

and

BB172: 5'-AAGCTTCTAGAGATCCCTCGACCTC-3' (SEQ ID NO:26).

Generation of an Intronless Human GALR2 Receptor

Human tissues may be screened by PCR, using primers that cross the intron, to identify cDNA sources that express the intronless form. An intronless hGALR2 clone may be obtained using an approach similar to that used to obtain an intronless rGALR2 clone (_infra_). Alternatively, one may use restriction enzymes to remove the intron and some adjacent coding region from BO29, and then replace the removed coding region by inserting a restriction enzyme-digested PCR fragment amplified from a tissue shown to express the intronless form of the receptor.

Human hippocampus and human hypothalamus were each shown to express the intronless form. A full-length, intronless human GALR2 PCR product was amplified from human hippocampus, but was found to contain a single point mutation downstream from the intron splice site. Therefore, an _Eco_RI/_Sty_I restriction digest fragment, containing 11 bp of 5'UT and the first 557 bp of hGalR2 coding region, was ligated to a _Sty_I restriction digest fragment, containing bp 558-1164 of the coding region and 182 bp of 3' UT, which was isolated from the intron-containing hGalR2 clone (BO29). The ligation product, comprising the entire intronless form of the human GALR2

receptor, was subcloned into the vector pEXJ and designated BO39.

Northern Blots

Human brain multiple tissue northern blots (MTN blots II and III, Clontech, Palo Alto, CA) carrying mRNA purified from various human brain areas may be hybridized according to the manufacturers' specifications.

Rat multiple tissue northern blots including multiple brain tissue blots (rat MTN blot, Clontech, Palo Alto, CA) carrying mRNA purified from various rat tissues also may be hybridized at high stringency according to the manufacturer's specifications.

RT-PCR analyses of GALR2 mRNA

Tissues may be homogenized and total RNA extracted using the guanidine isothiocyanate/CsCl cushion method. RNA may then be treated with DNase to remove any contaminating genomic DNA. cDNA may be prepared from total RNA with random hexanucleotide primers using the reverse transcriptase Superscript II (BRL, Gaithersburg, MD). First strand cDNA (about 250 ng of total RNA) may be amplified for example, in a 50 μL PCR reaction mixture (200 μM dNTPs final concentration) and 1 μM appropriate primers, using an appropriate thermal cycling program.

The PCR products may be run on a 1.5% agarose gel and transferred to charged nylon membranes (Zetaprobe GT, BioRad), and analyzed as Southern blots. GALR2 primers will be screened for the absence of cross-reactivity with the other galanin receptors. Filters may be hybridized with radiolabeled probes and washed under high stringency. Labeled PCR products may be visualized on X-ray film. Similar PCR and Southern blot analyses may be conducted with primers and probes, e.g., 1B15, directed to the housekeeping gene, glyceraldehyde phosphate

dehydrogenase (Clontech, Palo Alto, CA), to normalize the amount of cDNA used from the different tissues.

RT PCR of rat brain tissues was carried out using total
5   or poly A⁺ RNA (1.5 μg or 0.5 μg, respectively) isolated from various rat brain regions and converted to cDNA using Superscript II (BRL, Gaithersburg, MD) reverse transcriptase with random priming. The cDNAs were used as templates for PCR amplification of GALR2 using
10   specific GALR2 primers. PCR products were separated on an agarose gel by electrophoresis and blotted to a charged nylon membrane.

Isolation of the Intronless Rat GALR2
15   RT-PCR analysis of various rat brain regions (Figure 5) was carried out using primers representing N- and C- termini of rat GALR2 (supra). The forward and reverse primers comprised nucleotides 1-23 and 1087-1110, respectively, of the intronless rat GALR2 sequence (SEQ.
20   ID No. 7). The PCR products were separated by agarose gel electrophoresis, blotted, and hybridized with an oligonucleotide probe designed to the predicted 5/6 loop of GALR2 (nucleotides 651-695, SEQ. ID No. 7). This analysis indicated the presence of both intron-containing
25   and intronless forms of rat GALR2 in brain. In order to choose an appropriate tissue source from which to isolate the intronless form, a similar PCR analysis on RNA from a variety of rat tissues was carried out. Based on the size of the products determined by agarose gel
30   electrophoresis (data not shown), rat heart was chosen as a potential source of intronless GALR2 RNA. To isolate the intronless GALR2, PCR primers similar to those used above but containing restriction enzyme sites to facilitate subcloning and a Kozak consensus for
35   translation initiation (KS-1550 and KS-1551, see below) were used to amplify rat GALR2 from rat heart RNA by PCR (after conversion of the RNA to first strand cDNA by

standard methods). A PCR product of the correct size was
isolated from an agarose gel and then reamplified using
the same primers to increase yield. The products were
digested with the appropriate restriction enzymes to
5   produce cohesive ends (EcoRI and Xba I), ligated into the
expression vector EXJ.RH and transformed into E.coli.
The resulting colonies were transferred to nitrocellulose
membranes and hybridized with an oligonucleotide probe to
the predicted 2/3 loop of rat GALR2 (nucleotides 259-303,
10   SEQ. ID No. 7). A single hybridizing colony was found by
subsequent analysis to contain the intronless rat GALR2
cDNA.

Primers used:
15   Forward primer, KS-1550: 5'-ACGGAATTCGACATGAATGGCTCCGGCA
(SEQ. ID No. 27)

Reverse Primer, KS-1551:
5'-GCTCTAGAGCCCCTTTGGTCCTTTAACAAGCCGG (SEQ. ID No. 28)
20

Production of Recombinant Baculovirus
The coding region of GALR2 may be subcloned into
pBlueBacIII into existing restriction sites, or sites
engineered into sequences 5' and 3' to the coding region
25   of GALR2, for example, a 5' BamHI site and a 3' EcoRI
site. To generate baculovirus, 0.5 μg of viral DNA
(BaculoGold) and 3 μg of GALR2 construct may be co-
transfected into 2 x 10^6 Spodoptera fruqiperda insect Sf9
cells by the calcium phosphate co-precipitation method,
30   as outlined in by Pharmingen (in "Baculovirus Expression
Vector System: Procedures and Methods Manual"). The cells
then are incubated for 5 days at 27°C.

The supernatant of the co-transfection plate may be
35   collected by centrifugation and the recombinant virus
plaque purified. The procedure to infect cells with
virus, to prepare stocks of virus and to titer the virus

stocks are as described in Pharmingen's manual.

Cell Culture

COS-7 cells are grown on 150 mm plates in DMEM with
5   supplements (Dulbecco's Modified Eagle Medium with 10%
bovine calf serum, 4 mM glutamine, 100 units/mL
penicillin/100 μg/mL streptomycin) at 37°C, 5% CO_2. Stock
plates of COS-7 cells are trypsinized and split 1:6 every
3-4 days. Human embryonic kidney 293 cells are grown on
10   150 mm plates in D-MEM with supplements (minimal
essential medium) with Hanks' salts and supplements
(Dulbecco's Modified Eagle Medium with 10% bovine calf
serum, 4 mM glutamine, 100 units/mL penicillin/100 μg/mL
streptomycin) at 37°C, 5% CO_2. Stock plates of 293 cells
15   are trypsinized and split 1:6 every 3-4 days. Mouse
fibroblast LM(tk-) cells are grown on 150 mm plates in D-
MEM with supplements (Dulbecco's Modified Eagle Medium
with 10% bovine calf serum, 4 mM glutamine, 100 units/mL
penicillin/100 μg/mL streptomycin) at 37°C, 5% CO_2. Stock
20   plates of LM(tk-) cells are trypsinized and split 1:10
every 3-4 days. Chinese hamster ovary (CHO) cells were
grown on 150 mm plates in HAM's F-12 medium with
supplements (10% bovine calf serum, 4 mM L-glutamine and
100 units/mL penicillin/100 ug/ml streptomycin) at 37°C,
25   5% CO2. Stock plates of CHO cells were trypsinized and
split 1:8 every 3-4 days.

LM(tk-) cells stably transfected with the GALR2 receptor
may be routinely converted from an adherent monolayer to
30   a viable suspension. Adherent cells are harvested with
trypsin at the point of confluence, resuspended in a
minimal volume of complete DMEM for a cell count, and
further diluted to a concentration of 10^6 cells/mL in
suspension media (10% bovine calf serum, 10% 10X Medium
35   199 (Gibco), 9 mM $NaHCO_3$, 25 mM glucose, 2 mM L-glutamine,
100 units/mL penicillin/100 μg/mL streptomycin, and 0.05%
methyl cellulose). Cell suspensions are maintained in a

- 80 -

shaking incubator at 37°C, 5% CO_2 for 24 hours. Membranes harvested from cells grown in this manner may be stored as large, uniform batches in liquid nitrogen. Alternatively, cells may be returned to adherent cell culture in complete DMEM by distribution into 96-well microtiter plates coated with poly-D-lysine (0.01 mg/mL) followed by incubation at 37°C, 5% CO_2 for 24 hours. Cells prepared in this manner generally yield a robust and reliable response in cAMP radio-immunoassays as further described hereinbelow.

Mouse embryonic fibroblast NIH-3T3 cells are grown on 150 mm plates in Dulbecco's Modified Eagle Medium (DMEM) with supplements (10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100 µg/mL streptomycin) at 37°C, 5% CO2. Stock plates of NIH-3T3 cells are trypsinized and split 1:15 every 3-4 days.

Sf9 and Sf21 cells are grown in monolayers on 150 mm tissue culture dishes in TMN-FH media supplemented with 10% fetal calf serum, at 27°C, no CO_2. High Five insect cells are grown on 150 mm tissue culture dishes in Ex-Cell 400™ medium supplemented with L-Glutamine, also at 27°C, no CO_2.

Transfection
All receptor subtypes studied may be transiently transfected into COS-7 cells by the DEAE-dextran method, using 1 µg of DNA /10^6 cells (Cullen, 1987). In addition, Schneider 2 Drosophila cells may be cotransfected with vectors containing the receptor gene, under control of a promoter which is active in insect cells, and a selectable resistance gene, eg., the G418 resistant neomycin gene, for expression of the galanin receptor.

Stable Transfection
The GALR2 receptor may be co-transfected with a G-418

- 81 -

resistant gene into the human embryonic kidney 293 cell
line by a calcium phosphate transfection method (Cullen,
1987). GALR1 receptors were expressed in cells using
methods well-known in the art. Stably transfected cells
are selected with G-418. GALR2 receptors may be similarly
transfected into mouse fibroblast LM(tk-) cells, Chinese
hamster   ovary   (CHO)   cells   and   NIH-3T3   cells.
Transfection of LM(tk-) cells with the plasmid K985 and
subsequent selection with G-418 resulted in the LM(tk-)
cell line L-rGALR2-8 (ATCC Accession No. CRL-12074),
which stably expresses the rat GALR2 receptor. A similar
procedure   was   used   to   transfect   LM(tk-)   cells   with
plasmid K1045 (intronless rat GALR2 receptor construct)
resulting   in   the   LM(tk-)   cell   line   L-rGALR4-I   (ATCC
Accession No. CRL-12223). In addition, this procedure
was used to transfect CHO cells with an intron-containing
plasmid to create a stably expressing rat GALR2 CHO cell
line, C-GalR2-79 (ATCC Accession No. _____).

Radioligand binding assays

Transfected cells from culture flasks were scraped into
5 ml of Tris-HCl, 5mM EDTA, pH 7.5, and lysed by
sonication. The cell lysates were centrifuged at 1000
rpm for 5 min. at 4°C, and the supernatant was
centrifuged at 30,000 x g for 20 min. at 4°C. The pellet
was suspended in binding buffer (50mM Tris-HCl, 5mM MgSO$_4$,
1mM EDTA at pH 7.5 supplemented with 0.1% BSA, 2μg/ml
aprotinin,   0.5mg/ml   leupeptin,   and   10μg/ml
phosphoramidon). Optimal membrane suspension dilutions,
defined as the protein concentration required to bind
less than 10% of the added radioligand, were added to 96-
well polpropylene microtiter plates containing ^{125}I-labeled
peptide, non-labeled peptides and binding buffer to a
final volume of 250 μl. In equilibrium saturation
binding assays membrane preparations were incubated in
the presence of increasing concentrations (0.1nM to 4nM)
of [^{125}I]porcine galanin (specific activity 2200 Ci/mmol).

- 82 -

The binding affinities of the different galanin analogs
were determined in equilibrium competition binding
assays, using 0.1 nM [^{125}I]porcine galanin in the presence
of twelve different concentrations of the displacing
5   ligands. Binding reaction mixtures were incubated for 1
hr at 30°C, and the reaction was stopped by filtration
through GF/B filters treated with 0.5% polyethyleneimine,
using a cell harvester. Radioactivity was measured by
scintillation counting and data were analyzed by a
10   computerized non-linear regression program. Non-specific
binding was defined as the amount of radioactivity
remaining after incubation of membrane protein in the
presence of 100nM of unlabeled porcine galanin. Protein
concentration was measured by the Bradford method using
15   Bio-Rad Reagent, with bovine serum albumin as a standard.

Binding assays involving the rat GALR3 receptor are
conducted at room temperature for 120 min. in binding
buffer. Leupeptin, aprotonin and phosphoramidon are
20   omitted from rat GALR3 assays while bacitracin is added
to 0.1%. Nonspecific binding is defined in the presence
of 1 μM porcine galanin. Cells transiently or stably
expressing GALR3 receptors are produced using
transfection methods which are well-known in the art,
25   examples of which are provided herein (supra). The rat
GALR3 receptor may be expressed using plasmid K1086,
deposited on October 8, 1996, with the ATCC, 12301
Parklawn Drive, Rockville, Maryland 20852, U.S.A under
the Budapest Treaty on the International Recognition of
30   the Deposit of Microorganisms for the Purposes of Patent
Procedure, and was accorded ATCC Accession No. 97747.
Another plasmid expressing the rat GALR3 receptor is
plasmid pEXJ-rGALR3t, deposited with the ATCC under the
Budapest Treaty on December 17, 1996, and accorded ATCC
35   Accession No. 97826. The human GALR3 receptor may be
expressed using plasmid pEXJ-hGALR3, also deposited with
the ATCC under the Budapest Treaty on December 17, 1996,

- 83 -

and accorded ATCC Accession No. 97827. Cells stably expressing the GALR3 receptors may be used in functional assays well known in the art, examples of which are provided herein (infra).

Functional Assays
Cyclic AMP (cAMP) formation

The receptor-mediated inhibition of cyclic AMP (cAMP) formation may be assayed in LM(tk-) cells expressing the rat GALR1 and GALR2 receptors. Cells were plated in 96-well plates and incubated in Dulbecco's phosphate buffered saline (PBS) supplemented with 10 mM HEPES, 5mM theophylline, 2μg/ml aprotinin, 0.5mg/ml leupeptin, and 10μg/ml phosphoramidon for 20 min at 37°C, in 5% CO_2. Galanin or the test compounds were added and incubated for an additional 10 min at 37°C. The medium was aspirated and the reaction was stopped by the addition of 100 mM HCl. The plates were stored at 4°C for 15 min, and the cAMP content in the stopping solution was measured by radioimmunoassay. Radioactivity was quantified using a gamma counter equipped with data reduction software.

Functional assay experiments were also performed using stably transfected cells seeded into 96-well microtiter plates and cultured until confluent. To reduce the potential for receptor desensitization, the serum component of the media was reduced to 1.5% for 4 to 16 hours before the assay. Cells were washed in Hank's buffered saline, or HBS (150 mM NaCl, 20 mM HEPES, 1 mM $CaCl_2$, 5 mM KCl, 1 mM $MgCl$, and 10 mM glucose) supplemented with 0.1% bovine serum albumin plus 5 mM theophylline and pre-equilibrated in the same solution for 20 min at 37°C in 5% CO_2. Cells were then incubated 5 min with 10 μM forskolin and various concentrations of receptor-selective ligands. The assay was terminated by the removal of HBS and acidification of the cells with

- 84 -

100 mM HCl.   Intracellular cAMP was extracted and
quantified with a modified version of a magnetic bead-
based radioimmunoassay (Advanced Magnetics, Cambridge,
MA). The final antigen/antibody complex was separated
from free ^{125}I-cAMP by vacuum filtration through a PVDF
filter in a microtiter plate (Millipore, Bedford, MA).
Filters were punched and counted for ^{125}I in a Packard
gamma counter. Functional studies of the rat GALR1
receptor in LMTK- cells were performed as previously
described above except that leupeptin, aprotinin and
phosphoramidon were omitted from the assay, and cells
were stimulated with forskolin plus peptides for a period
of 5 min.

Arachidonic Acid Release

CHO cells stably transfected with the rat GALR2 receptor
were seeded into 96 well plates and grown for 3 days in
HAM's F-12 with supplements. ^{3}H-arachidonic acid
(specific activity = 0.75 uCi/ml) was delivered as a 100
ul aliquot to each well and samples were incubated at 37°
C, 5% CO_2 for 18 hours. The labeled cells were washed
three times with 200 ul HAM's F-12. The wells were then
filled with medium (200 uL) and the assay was initiated
with the addition of peptides or buffer (22 uL). Cells
were incubated for 30 min at 37°C, 5% CO2. Supernatants
were transferred to a microtiter plate and evaporated to
dryness at 75°C in a vacuum oven. Samples were then
dissolved and resuspended in 25 uL distilled water.
Scintillant (300 uL) was added to each well and samples
were counted for ^{3}H in a Trilux plate reader. Data were
analyzed using nonlinear regression and statistical
techniques available in the GraphPAD Prism package (San
Diego, CA).

Intracellular calcium mobilization

The intracellular free calcium concentration may be
measured by microspectroflourometry using the fluorescent

indicator dye Fura-2/AM (Bush et al. 1991). Cells stably
transfected with GALR2 are seeded onto a 35 mm culture
dish containing a glass coverslip insert. Cells are
washed with HBS and loaded with 100 μL of Fura-2/AM (10

5   μM) for 20 to 40 min. After washing with HBS to remove
the Fura-2/AM solution, cells are equilibrated in HBS for
10 to 20 min. Cells are then visualized under the 40X
objective of a Leitz Fluovert FS microscope and
fluorescence emission is determined at 510 nM with

10   excitation wavelengths alternating between 340 nM and 380
nM. Raw fluorescence data are converted to calcium
concentrations using standard calcium concentration
curves and software analysis techniques.

15   <u>Phosphoinositide metabolism</u>
LM(tk-) cells stably expressing the rat GALR2 receptor
cDNA were plated in 96-well plates and grown to
confluence. The day before the assay the growth medium
was changed to 100 μl of medium containing 1% serum and

20   0.5 μCi [^{3}H]myo-inositol, and the plates were incubated
overnight in a CO_2 incubator (5%$_2$ CO at 37^{0}C).
Alternatively, arachidonic acid release may be measured
if [^{3}H]arachidonic acid is substituted for the [^{3}H]myo-
inositol. Immediately before the assay, the medium was

25   removed and replaced by 200 μL of PBS containing 10 mM
LiCl, and the cells were equilibrated with the new medium
for 20 min. During this interval cells were also
equilibrated with the antagonist, added as a 10 μL
aliquot of a 20-fold concentrated solution in PBS. The

30   [^{3}H]inositol-phosphates accumulation from inositol
phospholipid metabolism was started by adding 10 μL of a
solution containing the agonist. To the first well 10 μL
were added to measure basal accumulation, and 11
different concentrations of agonist were assayed in the

35   following 11 wells of each plate row. All assays were
performed in duplicate by repeating the same additions in
two consecutive plate rows. The plates were incubated in

- 86 -

a CO_2 incubator for 1 hr. The reaction was terminated by adding 15 μl of 50% v/v trichloroacetic acid (TCA), followed by a 40 min. incubation at 4^0C. After neutralizing TCA with 40 μl of 1M Tris, the content of the wells was transferred to a Multiscreen HV filter plate (Millipore) containing Dowex AG1-X8 (200-400 mesh, formate form). The filter plates were prepared adding 200 μL of Dowex AG1-X8 suspension (50% v/v, water: resin) to each well. The filter plates were placed on a vacuum manifold to wash or elute the resin bed. Each well was washed 2 times with 200 μL of water, followed by 2 x 200 μL of 5mM sodium tetraborate/60 mM ammonium formate. The [^{3}H]IPs were eluted into empty 96-well plates with 200 μl of 1.2 M ammonium formate/0.1 formic acid. The content of the wells was added to 3 mls of scintillation cocktail, and the radioactivity was determined by liquid scintillation counting.

Functional assays using GALR3 receptors are performed similarly.

It is to be understood that the cell lines described herein are merely illustrative of the methods used to evaluate the binding and function of the galanin receptors of the present invention, and that other suitable cells may be used in the assays described herein.

Functional responses in oocytes expressing GalR2

Female *Xenopus laevis* (Xenopus-1, Ann Arbor, MI) were anesthetized in 0.2% tricain (3-aminobenzoic acid ethyl ester, Sigma Chemical Corp.) and a portion of ovary was removed using aseptic technique (Quick and Lester, 1994). Oocytes were defolliculated using 2 mg/ml collagenase (Worthington Biochemical Corp., Freehold, NJ) in a solution containing 87.5 mM NaCl, 2 mM KCl, 2 mM $MgCl_2$ and 5 mM HEPES, pH 7.5. Oocytes were injected (Nanoject,

- 87 -

Drummond Scientific, Broomall, PA) with 50 nL of rat
GalR2 mRNA or other mRNA for use as a negative control.
RNA was prepared by linearization of the plasmid
(pBluescript) containing the entire coding region of the
5   GalR2 cDNA, followed by _in vitro_ transcription using the
T7 polymerase ("MessageMachine", Ambion). Alternatively,
mRNA may be translated from a template generated by PCR,
incorporating a T7 promoter. Oocytes were incubated at
16° on a rotating platform for 3-8 days post-injection.
10   Dual electrode voltage clamp ("GeneClamp", Axon
Instruments Inc., Foster City, CA) was performed using 3
M KCl-filled glass microelectrodes having resistances of
1-3 Mohms. Unless otherwise specified, oocytes were
clamped at a holding potential of -80 mV. During
15   recordings, oocytes are bathed in continuously flowing
(2-5 ml/min) medium containing 96 mM NaCl, 2 mM KCl, 2 mM
$CaCl_2$, 2 mM $MgCl_2$, 5 mM HEPES, pH 7.5 (ND96). Drugs are
applied by switching from a series of gravity fed
perfusion lines.

20

The human GALR2 receptor and GALR3 receptors may be
studied functionally using similar methods.

Galanin Receptor Autoradiography

25   Male Sprague-Dawley rats (Charles River, Wilmington,
Massachusetts) were euthanized using CO_2, decapitated, and
their brains immediately removed and frozen on dry ice.
Tissue sections were cut at 20 μm using a cryostat and
thaw mounted onto gelatin coated slides. Tissues were
30   preincubated in two 10 minute changes of 50 mM Tris-HCl
buffer pH 7.4, containing 5 mM $MgSO_4$ and 2 mM EGTA
(Sigma). The radioligand binding was carried out in the
same buffer, which also contained 0.1% bovine serum
albumin, 0.02% aprotinin, 0.031% leupeptin, 0.1%
35   phosphoramidate (Boehringer Mannheim), and 0.1 nM
[^{125}I]porcine galanin (specific activity 2200 Ci/mmol, NEN)
for 1 hour at 22°C. Nonspecific binding was determined

- 88 -

in the presence of 5 μM porcine galanin (Bachem). As [D-Trp^2]galanin$_{(1-29)}$ was shown to be selective for the cloned GA1R2 receptor (<u>infra</u>), a 60 nM concentration of this peptide was used to displace [^{125}I]galanin binding from the rat brain tissue sections. The use of this concentration was based on the binding data, which showed the affinity of [D-Trp^2]galanin$_{(1-29)}$ to be 6 nM at the GALR2 receptor, and 3 μM at the GALR1 receptor. In general, a 10X concentration of the blocking ligand is sufficient to remove 100% of the targeted receptor, while leaving the GALR1 receptor unaffected. After incubation, tissues were dipped twice in ice-cold Tris-HCl buffer (4°C), followed by a 5 minute wash in ice-cold Tris-HCl buffer (4°C), then dipped twice in ice-cold deionized water to remove the salts. Sections were placed in X-ray cassettes and apposed to Dupont Cronex MRF 34 Film for 5 days. Films were developed using a Kodak M35A Processor.

<u>Tissue preparation for neuroanatomical studies</u>

Male Sprague-Dawley rats (Charles River) are decapitated and the brains rapidly removed and frozen in isopentane. Coronal sections are cut at 11 μm on a cryostat and thaw-mounted onto poly-L-lysine coated slides and stored at -80°C until use. Prior to hybridization, tissues are fixed in 4% paraformaldehyde, treated with 5 mM dithiothreitol, acetylated in 0.1 M triethanolamine containing 0.25% acetic anhydride, delipidated with chloroform, and dehydrated in graded ethanols.

<u>Probes</u>

Oligonucleotide probes employed to characterize the distribution of the rat GALR2 receptor mRNA may be synthesized, for example, on a Millipore Expedite 8909 Nucleic Acid Synthesis System. The probes are then lyophilized, reconstituted in sterile water, and purified on a 12% polyacrylamide denaturing gel. The purified probes are again reconstituted to a concentration of 100

ng/μL, and stored at -20°C. Probe sequences may include
DNA or RNA which is complementary to the mRNA which
encodes the GALR2 receptor.

Localization of GALR2 mRNA: In situ hybridization
Animals
Timed-pregnant female Sprague-Dawley rats were puchased
from Charles River. The day of birth for each litter was
designated as postnatal day 0 (P0). Brains were removed
from pups on P0, P3, P5, P8, P10, P15, P20, and P25. The
brains from the mothers were also removed and used as the
adult comparison. All brains were sectioned in the
coronal plane at 11 μm and the sections thaw-mounted on
to poly-l-lysine coated microscope slides. The sections
were then used for *in situ* hybridization histochemistry
as described below.

Tissue preparation
Prior to hybridization, tissues were fixed in 4%
paraformaldehyde, treated with 5 mM dithiothreitol,
acetylated in 0.1 M triethanolamine containing 0.25%
acetic anhydride, delipidated with chloroform, and
dehydrated in graded ethanols. The sections were
prehybridized for one hour at 40°C in hybridization
buffer, which consisted of 50% formamide, 4X sodium
citrate buffer (1X SSC = 0.15 M NaCl and 0.015 M sodium
citrate), 1X Denhardt's solution (0.2%
polyvinylpyrrolidine, 0.2% Ficoll, 0.2% bovine serum
albumin), 50 mM dithiothreitol, 0.5 mg/ml salmon sperm
DNA, 0.5 mg/ml yeast tRNA, and 10% dextran sulfate.

In Situ Hybridization
32mer oligonucleotide probes complementary to nucleotides
261-292 of the GALR2 mRNA were synthesized, purified, and
3'-end labeled with ^{35}S-dATP (1200 Ci/mmol, New England
Nuclear, Boston, MA) to a specific activity of 10^9 dpm/μg
using terminal deoxynucleotidyl transferase (Boehringer

- 90 -

Mannheim; Indianapolis, IN). The radiolabeled probes
were purified on Biospin 6 chromatography columns (Bio-
Rad; Richmond, CA), and diluted in the hybridization
buffer described above to a concentration of 1.5×10^4

5   cpm/μl. One hundred μl of the radiolabeled probe was
applied to each section, which was then covered with a
Parafilm coverslip. Hybridization was carried out
overnight in humid chambers at 40 to 55°C. The following
day the sections were washed in two changes of 2X SSC for

10   one hour at room temperature, in 2X SSC for 30 min at 50-
60°C, and finally in 0.1X SSC for 30 min at room
temperature. Tissues were dehydrated in graded ethanols
and apposed to Kodak XAR-5 film for 2 weeks at -20°C, then
dipped in Kodak NTB3 autoradiography emulsion diluted 1:1

15   with 0.2% glycerol water. After exposure at 4°C for 4
weeks, the slides were developed in Kodak D-19 developer,
fixed, and counterstained with hematoxylin and eosin.

Localization of GALR2 mRNA: Ribonuclease protection assay
20   (RPA)
Development of probes
A cDNA fragment encoding a 467 BP fragment of the rGAL R2
was subcloned into a pBluescript plasmid vector. This
construct was linearized with Xba I or Sal I. T3 and T7

25   RNA polymerases were used to synthesize the sense and
antisense strands of RNA respectively. Full-length RNA
transcripts were obtained using a full-length cDNA
construct in the same vector.

30   A probe coding for rat glyceraldehyde 3-phosphate
dehydrogenase (GAPDH) gene, a constitutively expressed
protein, was used concurrently. GAPDH is expressed at a
relatively constant level in most tissue and its
detection was used to compare expression levels of the

35   rGalR2 gene in different tissues.

RNA Extraction

RNA was isolated from rat peripheral tissue as well as regions of the CNS using a LiCl precipitation protocol (Cathala et al.. 1983). Tissue was homogenized in 5M guanidine isothiocyanate, 50mM TRIS, 10 mM EDTA, using 7
5   ml of lysis buffer/gram tissue. 4M LiCl were added (7ml/ml homogenate) and the mixture were stored at 4°C for 24-48 hours. Homogenates were centrifuged and the pellets were resuspended in 3M LiCl, and centrifuged again. The pellets were resuspended in 0.1% sodium
10 dodecyl   sulfate   (SDS),   extracted   in phenol:chloroform:isoamyl alcohol (24:24:1) and the RNA ethanol precipitated. Yield and relative purity were assessed by measuring absorbance A_{260}/A_{280}.

15 <u>Synthesis of probes</u>

rGALR2 and GAPDH cDNA sequences preceded by phage polymerase promoter sequences were used to synthesize radiolabeled riboprobes. Conditions for the synthesis of riboprobes were: 1-2 μl linearized template (1μg/μl),
20 1μl of ATP, GTP, UTP (10 mM each), 2 μl dithiothreitol (0.1 M), 20 units RNAsin RNAse inhibitor, 1-2 μl (15-20 units/μl) RNA polymerase, 4 μl transcription buffer (Promega Corp.), and 5 μl α^{32}P-CTP (specific activity 800Ci/mmol). 0.1 mM CTP (0.02-1.0 μl) were added to the
25 reactions, and the volume were adjusted to 20 μl with DEPC-treated water. Labeling reactions were incubated at 38°C for 90 min, after which 2 units of RQ1 RNAse-free DNAse (Promega Corp.) were added to digest the template. The riboprobes were separated from unincorporated
30 nucleotide by a spun G-50 column (Select D G-50(RF); 5 Prime-3 Prime, Inc.). TCA precipitation and liquid scintillation spectrometry were used to measure the amount of label incorporated into the probe. A fraction of all riboprobes synthesized were size-fractionated on
35 0.4 mm thick 5% acrylamide sequencing gels and autoradiographed to confirm that the probes synthesized were full-length and not degraded.

<u>Solution hybridization/ribonuclease protection assay</u>

For solution hybridization 2-15 μg of total RNA isolated
from tissues were used. Sense RNA synthesized using the
full-length coding sequence of the rGalR2 was used to
5   characterize specific hybridization. Negative controls
consisted of 30 μg transfer RNA (tRNA) or no tissue
blanks. All samples were placed in 1.5-ml microfuge
tubes and vacuum dried. Hybridization buffer (40 μl of
400 mM NaCl, 20 mM Tris, pH 6.4, 2 mM EDTA, in 80%
10   formamide) containing 0.25-1.0 X 10^6 counts of each probe
were added to each tube. Samples were heated at 90°C for
15 min, after which the temperature were lowered to 45°C
for hybridization.

15   After hybridization for 14-18 hr, the RNA/probe mixtures
were digested with RNAse A (Sigma) and RNAse T1 (Bethesda
Research Labs). A mixture of 2.0 μg RNAse A and 1000
units of RNAse T1 in a buffer containing 330 mM NaCl, 10
mM Tris (pH 8.0) and 5 mM EDTA (400 μl) was added to each
20   sample and incubated for 90 min at room temperature.
After digestion with RNAses, 20 μl of 10% SDS and 50 μg
proteinase K were added to each tube and incubated at
37°C for 15 min. Samples were then extracted with
phenol/chloroform:isoamyl alcohol and precipitated in 2
25   volumes of ethanol for 1 hr at -70°C. tRNA was added to
each tube (30 mg) as a carrier to facilitate
precipitation. Following precipitation, samples were
centrifuged, washed with cold 70% ethanol, and vacuum
dried. Samples were dissolved in formamide loading
30   buffer and size-fractionated on a urea/acrylamide
sequencing gel (7.6 M urea, 6% acrylamide in Tris-borate-
EDTA). Gels were dried and apposed to Kodak XAR-5 x-ray
film.

35   <u>In vivo methods</u>

The effects of galanin, galanin derivatives, and related
peptides   and   compounds   were   evaluated   by

- 93 -

intracerebroventricular (i.c.v.) injection of the peptide
or compound followed by measurement of food intake in the
animal. Measurement of food intake was performed for 3
hours after injection, but other protocols may also be
used. Saline was injected as a control, but it is
understood that other vehicles may be required as
controls for some peptides and compounds. In order to
determine whether a compound is a GALR2 antagonist, food
intake in rats may be stimulated by administration of
(for example) the GALR2-selective peptide agonist [D-
Trp$_2$]-galanin $_{(1-29)}$ through an intracerebroventricular
(i.c.v.) cannula. A preferred anatomic location for
injection is the hypothalamus, in particular, the
paraventricular nucleus. Methods of cannulation and food
intake measurements are well-known in the art, as are
i.c.v. modes of administration (Kyrkouli et al., 1990,
Ogren et al., 1992). To determine whether a compound
reduces [D-Trp$_2$]-galanin $_{(1-29)}$ stimulated food intake, the
compound may be administered either simultaneously with
the peptide, or separately, either through cannula, or by
subcutaneous, intramuscular, or intraperitoneal
injection, or more preferably, orally.

Materials

Cell culture media and supplements are from Specialty
Media (Lavallette, NJ). Cell culture plates (150 mm and
96-well microtiter) are from Corning (Corning, NY). Sf9,
Sf21, and High Five insect cells, as well as the
baculovirus transfer plasmid, pBlueBacIII™, are purchased
from Invitrogen (San Diego, CA). TMN-FH insect medium
complemented with 10% fetal calf serum, and the
baculovirus DNA, BaculoGold™, is obtained from Pharmingen
(San Diego, CA.). Ex-Cell 400™ medium with L-Glutamine
is purchased from JRH Scientific. Polypropylene 96-well
microtiter plates are from Co-star (Cambridge, MA). All
radioligands are from New England Nuclear (Boston, MA).

Galanin and related peptide analogs were either from
Bachem California (Torrance, CA), Peninsula (Belmont,
CA); or were synthesized by custom order from Chiron
Mimotopes Peptide Systems (San Diego, CA).

5

Bio-Rad Reagent was from Bio-Rad (Hercules, CA). Bovine
serum albumin (ultra-fat free, A-7511) was from Sigma
(St. Louis. MO). All other materials were reagent grade.

Experimental Results

Isolation of a GALR2 cDNA from rat hypothalamus

In order to clone additional members of the galanin
5   receptor family, an expression cloning strategy based on
the potential presence of multiple galanin receptors in
hypothalamus was designed. Although recent evidence
indicated that GALR1 receptor mRNA was present in rat
hypothalamus (Gustafson et al., 1996; Parker et al.,
10   1995),   not   all   aspects   of   the   cloned   GALR1
pharmacological profile match that observed for galanin-
mediated feeding (Crawley et al., 1993). These results
suggested that the regulation of galanin-induced feeding
may not be explained by the presence of only GALR1 in the
15   rat hypothalamus.

A randomly-primed cDNA expression library was constructed
from   rat   hypothalamus   and   screened   by   radioligand
binding/photoemulsion   detection   using   [^{125}I]-porcine
20   galanin. The library consisted of 584 pools containing
about 5,000 primary clones/pool for a total of about 3
million clones with an average insert size of 2.2 kb.
Pools positive for rat GALR1 (about 110) were eliminated
from the screen.   Remaining pools were screened for
25   radioligand binding using 1 nM [^{125}I]-porcine galanin;
slides were inspected for positive cells by direct
microscopic examination. One positive pool (J126) was
subdivided into 96 pools of about 90 clones each and
rescreened for galanin binding. Preliminary pharmacology
30   carried out on the positive subpool J126-10 indicated
that the [^{125}I]-porcine galanin binding was not sensitive
to inhibition by galanin 3-29. 400 individual colonies
of a positive pool (J26-10) were then screened to find
two single purified cDNA clones. J126-10-334 was chosen
35   for further analysis and designated K985. PCR analysis
using three independent GALR1 primer sets (see Methods;
data not shown) confirmed that the newly isolated cDNA

- 96 -

was distinct from GALR1 and thus encoded a new galanin
receptor subtype, termed GALR2.

The isolated clone K985 carries a 3.8 kb insert.
Sequence analysis of this cDNA revealed a complete coding
region for a novel receptor protein which we term GALR2
(see Figures 1 and 2). Searches of GenEMBL databases
indicated that the sequence was novel, and that the most
similar sequence was that of the galanin receptor GALR1,
followed by other G protein-coupled receptors (GPCR).
The nucleotide and deduced amino acid sequences are shown
in Figures 1 and 2, respectively. The nucleotide
sequence of the coding region is ~56% identical to rat
GALR1 and ~54% identical to human GALR1 and encodes a 372
amino acid protein with 38% and 40% amino acid identity
to rat and human GALR1, respectively. Hydropathy plots
of the predicted amino acid sequence reveal seven
hydrophobic regions that may represent transmembrane
domains (TMs, data not shown), typical of the G protein-
coupled receptor superfamily. In the putative TM
domains, GALR2 exhibits 48-49% amino acid identity with
rat and human GALR1. Like most GPCRs, the GALR2 receptor
contains consensus sequences for N-linked glycosylation
in the N-terminus (positions 2 and 11) as well as the
predicted extracellular loop between TMs IV and V. The
GALR2 receptor contains two highly conserved cysteine
residues in the first two extracellular loops that are
believed to form a disulfide bond stabilizing the
functional protein structure (Probst et al., 1992).
GALR2 shows five potential phosphorylation sites for
protein kinase C in positions 138, 210, 227, 319, and
364, and two cAMP- and cGMP-dependent protein kinase
phosphorylation sites in positions 232 and 316. It
should be noted that six out of the seven potential
phosphorylation sites are located in predicted
intracellular domains, and therefore could play a role in
regulating functional characteristics of the GALR2

receptor (Probst et al., 1992).

Within the GALR2 cDNA K985 (J126-10-334) isolated from the rat hypothalamus library, the coding region of GALR2 is interrupted by an intron of ~1 kb (Figures 3A, 3B, and 3C). A cDNA containing an intron may be produced by the action of reverse transcriptase on an incompletely spliced form of. messenger RNA. The heterologous expression of the complete protein product is not necessarily impeded by the presence of the intron in the coding region, because the intron can typically be spliced out prior to translation by the host cell machinery. In the case of the GALR2 cDNA, the location of the intron combined with clear consensus sequences for 5' and 3' splice junctions (Figures 3A and 3B) confirm that the intervening sequence represents an intron. As shown in Figure 3C, splicing of the intron at the indicated sites recreates an open reading frame within a highly conserved region of the GPCR family, at the end of TMIII (LDR/Y). It is of interest to note that several GPCRs have previously been reported to contain introns at this location, including the human dopamine D3, D4, and D5 receptors, the rat substance P receptor, and the human substance K receptor (Probst et al., 1992). In particular, the rat 5-HT$_7$ receptor (Shen et al., 1993) contains an intron in exactly the same location as is now reported for GALR2, within the AG/G codon for the highly conserved amino acid arginine at the end of TMIII (Figure 3C)..

To explore the possibility that incompletely or alternately spliced forms GALR2 mRNAs are present in the rat brain, RT-PCR using GALR2 PCR primers that are located in the coding region but that span the location of the intron was carried out. The sequences of the PCR primers are:

KS-1515 (Forward primer): 5'-CAAGGCTGTTCATTTCCTCATCTTTC
(loop between TMs II and III)(SEQ.ID No. 12).

KS-1499 (Reverse primer): 5'-TTGGAGACCAGAGCGTAAACGATGG
5   (end of TMVII)(SEQ.ID No. 13).

The PCR products were separated by gel electrophoresis,
blotted, and hybridized with a radiolabeled
oligonucleotide probe representing the predicted loop
10   between TMs V and VI. The sequence of the
oligonucleotide is:

KS-1540: 5'-AGTCGACCCGGTGACTGCAGGCTCAGGTTCCCAGCGCGCCAAACG
(SEQ. ID No. 14).

15

RT-PCR analysis of GALR2 mRNA from various rat brain
regions as described above indicates the existence of PCR
products that may represent both the intronless (spliced)
and intron-containing (incompletely spliced) forms of
20   GALR2 (Figure 5). In addition, PCR products intermediate
in size between intronless and intron-containing products
that hybridize at high stringency with the GALR2
oligonucleotide probe KS-1540 are present and may
represent additional variations in the GALR2 mRNA. One
25   mechanism that could generate such variations is
alternative splicing. These results suggest that
intronless transcripts exist in native tissue. A full-
length intronless cDNA encoding the rat GALR2 receptor
has been amplified and subcloned from rat heart RNA,
30   which when transiently or stably transfected into cells
binds galanin with high affinity.

Northern Blot Analysis of GALR2 mRNA

To define the size and distribution of the mRNA encoding
35   GALR2 Northern blot analysis of poly A+ RNA from various
rat tissues and brain regions was carried out. A ~1.2 kb
fragment of rat GALR2 containing the entire coding region

but not containing the intron (Figure 1) was radiolabeled
by random priming and used as a hybridization probe.
Northern blots containing rat poly A$^+$ RNA were hybridized
at high stringency and apposed to film. A single
5   transcript of ~1.8-2.0 kb is detected after a 4 day
exposure of the autoradiogram at -80°C using Kodak Biomax
MS film with one Biomax MS intensifying screen. Within
the brain, the highest levels of GALR2 mRNA appear in
hypothalamus (Figure 6A). Among various rat tissues, the
10   GALR2 transcript is widely but unevenly distributed:
GALR2 mRNA is observed in brain, lung, heart, spleen, and
kidney, with lighter bands in skeletal muscle, liver,
and testis (Figure 7A). Both Northern blots were
reprobed with 1B15 to confirm that similar amounts of
15   mRNA were present in each lane (Figures 6B and 7B).

Pharmacological characterization of GALR2

The pharmacology of GALR2 was studied in COS-7 cells
transiently transfected with the GALR2 cDNA, K985.
20   Membrane preparations of Cos-7 cells transfected with
K985 displayed specific binding to [^{125}I]porcine galanin.
Scatchard analysis of equilibrium saturation binding data
yielded a K_d = 150 pM with a B_{max} = 250 fmol/mg protein.
The pharmacological properties of the protein encoded by
25   the GALR2 cDNA were probed by measuring the binding
affinities of a series of galanin anologs, and compared
to those of the rat GALR1 receptor expressed in the same
host cell line. As shown in Table 1, both GALR1 and
GALR2 receptors showed a high affinity for galanin$_{(1-29)}$,
30   the physiological ligand of these receptors. Both
receptors also displayed high affinity for the truncated
analogs galanin$_{(1-16)}$ and galanin $_{(1-15)}$. Furthermore, the
binding of [^{125}I]porcine galanin to either GALR1 or GALR2
at concentrations up to 100μM was not displaced by
35   porcine galanin$_{(3-29)}$. However, the GALR2 receptor has 540-
and 4200-fold higher affinity for [D-Trp^2]porcine
galanin$_{(1-29)}$, and [D-Trp^2]galanin$_{1-16)}$, respectively, than

- 100 -

the GALR1 subtype.   Also, [Ala⁵]galanin$_{(1-16)}$,   and [Phe²]galanin$_{(1-15)}$ were moderately selective, with 15- and 17-fold greater affinities for the GALR2 receptor than for the GALR1 receptor subtype, respectively. [Ala⁹]galanin$_{(1-16)}$ was the only analog that was found to have the opposite selectivity, with 70-fold higher affinity for the GALR1 receptor than for the GALR2 receptor.   Interestingly, these two receptor subtypes showed no significant differences in their binding affinities for the chimeric galanin antagonists, galantide, C7, M32, M35, and M40.

In LM(tk-) cells stably expressing the rat GALR2 receptor cDNA, porcine galanin$_{(1-29)}$ was found to inhibit the formation of cyclic AMP induced by 10 μM forskolin. The effects of galanin were dose dependent with an EC_{50} = 0.26 ± 0.13 nM (n=3) (Figure 9A).   In the same cell line porcine galanin $_{(1-29)}$ stimulated the formation of [³H]inositol phosphates, with an EC_{50} =112 nM (Figure 9B). The phosphoinositide response mediated by the rat. GALR2 receptor suggests that this receptor can also couple to the intracellular calcium mobilization and diacylglycerol pathway.   However, the 400-fold lower EC $_{50}$ of porcine galanin $_{(1-29)}$ suggests that the GALR2 receptor couples with low efficiency to this signaling pathway.   In support of this notion stands the observation that porcine galanin $_{(1-29)}$ had no effect on intracellular calcium levels in COS-7 cells transfected with the cDNA encoding the rat GALR2 receptor.   Thus, the data presented herein suggest that the GALR2 receptor couples preferentially to G_{ialpha}, since the stimulation of phosphoinositide metabolism and intracllular calcium mobilization are a hallmark or receptors to the G_{qa} family of G-proteins. Furthermore, the data presented herein also indicate that the inhibition of cAMP formation, as well as the stimulation of phosphoinositide metabolism, can be used as functional assays to measure receptor activity in heterologous cell

systems expressing the rat GALR2 receptor.

In subsequent experiments, the inhibitory effect of rat
GALR2 receptor stimulation on forskolin-stimulated cAMP
accumulation in LM(tk-) cells could not be reproduced.
However, the same LM(tk-) cells yielded a reproducible PI
hydrolysis response (Table 4), and in independent binding
assays a B_{max} of 4000 fmol/mg protein and a K_d of 1.1 nM
when incubated with porcine ^{125}I-galanin. It is concluded
that in the cell lines studied thus far, the rat GalR2 is
coupled <u>primarily</u> to the activation of phospholipase C
and subsequent inositol phosphate metabolism, presumably
through Gq or a related G protein. The PI response was
evident as well in LM(tk-) cells stably transfected with
the rat GALR2 receptor cDNA lacking an intron in the
coding region (L-rGALR2I-4, see Table 4); membranes from
these cells were shown in an independent experiment to
bind porcine ^{125}I-galanin with a B_{max} of 4800 fmol/mg
membrane protein and a K_d of 0.2 nM.

The CHO cell line stably transfected with the rat GALR2
receptor (C-rGALR2-79) provided additional detail about
the binding and signalling properties of the receptor.
Membranes from stably transfected CHO cells were bound
saturably by porcine ^{125}I-galanin with a B_{max} of 520 fmol/mg
membrane protein and a K_d of 0.53 nM. Peptides displaced
the porcine ^{125}I-galanin (Table 5) with binding affinities
similar to those generated from transiently transfected
COS-7 cells (Table 1). Receptor stimulation resulted in
phosphatidyl inositol hydrolysis but had no effect on
cAMP accumulation, again supporting the proposal that the
rat GALR2 receptor is coupled primarily to phospholipase
C activation through Gq or a related G protein. It was
further demonstrated that rat GALR2 receptor activation
could be monitored by arachidonic acid release (Table 5).
Of interest, it was observed that the EC_{50} values from the
PI hydrolysis assays were larger than the K_i values from

- 102 -

binding assays whereas the EC_{50} values from the arachidonic acid assays were comparable to the binding data. One possibility suggested by these data is that the calcium release induced by inositol phosphate

5   metabolism leads to activation of phospholipase A2 and subsequently to the hydrolysis of arachidonic acid from membrane phospholipids. The lower EC_{50} values in the arachidonic acid assays may reflect an amplification process in the second messenger pathway, such that a

10   maximal arachidonic acid response occurs at submaximal calcium concentrations.

The stably transfected CHO cells were used to further explore the binding and signalling properties of the rat

15   GalR2 receptors (Table 6). The peptide binding profile was similar to that generated previously with transiently transfected COS-7 cells. Porcine, rat and human galanin bound with high affinity as did C-terminally truncated peptides as short as galanin 1-12.

20   Chimeric or putative "antagonist" peptides including C7, galantide, M32, M35 and M40 displayed relatively high binding affinity except for C7 (Ki = 47 nM). Galanin analogs containing D-Trp^2 (D-Trp^2-galanin 1-29 and D-Trp^2-galanin 1-16) retained measurable binding affinity (K_i =

25   41 and 110 nM, respectively). The N-terminally truncated peptide galanin 3-29 was inactive.

Selected peptides were subsequently tested in the arachidonic acid release assays. Peptides with

30   measurable EC_{50} values mimicked the maximal effect of rat galanin (1 μM) on arachidonic acid release and were classified as full agonists, including C7, galantide, M32, M35 and M40. The binding and functional profiles were in general agreement. Notable exceptions include

35   D-Trp^2-galanin 1-29, D-Trp^2-galanin 1-16, and C7, all of which generated larger K_i values vs. EC_{50} values; one possibility is that these peptides were less stable in

the binding assay vs. the functional assay. It is, therefore, concluded that the arachidonic acid release assay is useful for assessing peptide potency and intrinsic activity for the rat GalR2 receptor when stably expressed in CHO cells.

Peptides were further evaluated for their ability to selectively activate the rat GALR1 receptor (monitored in stably transfected LM(tk-) cells using the forskolin-stimulated cAMP accumulation assay) vs. the rat GALR2 (monitored in stably transfected CHO cells using the arachidonic acid release assay). Data are reported in Table 7. D-Trp^2-galanin was 8.5-fold less potent than galanin in the rat GALR2 functional assay but > 15000-fold less potent than galanin in the rat GALR1 functional assay. Similarly, D-Trp^2-galanin 1-16 was 38-fold less potent than galanin in the rat GALR2 functional assay but > 170,000-fold less potent than galanin in the rat GALR1 functional assay. It is concluded that D-Trp^2-galanin and analogous peptides may serve as useful tools with which to explore the function of GALR2 vs. GALR1 receptors in native tissues and physiological systems.

Feeding Assays

Rats were injected icv with either galanin, galanin derivatives, or saline. Cumulative food intake was measured over a period of 3 hours. Baseline food intake associated with the saline control was 1.5 gram. A maximal food intake of 6.81 grams was observed after a 10 nmole injection of galanin. The ED50 for galanin is estimated to be 1 nmole. M40 was also tested in this paradigm. M40 was able to mimic the effects of galanin, with a maximal food intake of 6.3 grams observed after a 50 nmol injection. The ED50 for M40 is estimated to be 20 nmoles.

Heterologous expression of GPCRs in Xenopus oocytes

- 104 -

Heterologous expression of GPCRs in Xenopus oocytes has been widely used to determine the identity of signaling pathways activated by agonist stimulation (Gundersen et al., 1983; Takahashi et al., 1987). Application of
5   porcine galanin (100-1000 nM) activates rapid inward currents in 36 of 46 oocytes injected with 5-50 pg rGalR2 mRNA (Fig. 13). Equimolar concentrations of C7 induces similar currents whereas galanin 3-29 is inactive (0/11 oocytes). Oocytes injected with buffer (ND96) alone or
10 5-HT1a receptor mRNA do not exhibit detectable (< 5 nA) responses to galanin (0/19). Current magnitudes in rGalR2 mRNA-injected oocytes range from small fluctuations of less than 50 nA (excluded from analysis) to large rapid currents (up to 3 μA) resembling those
15 activated by stimulation of other receptors (alpha1a receptors - data not shown) that are known to couple to IP3 release and stimulation of Cl- current from the resulting increase in intracellular free Ca^{++} (Takahashi et al., 1987). The currents stimulated by galanin in
20 oocytes expressing rGalR2 are most likely mediated by the endogenous calcium-activated Cl$^-$ channel (Gunderson et al., 1983) because they are blocked in oocytes injected with 50 nl of 10 mM EGTA (5/5) and they display a current-voltage relation that exhibits outward
25 rectification and a reversal potential of approximately -15 mV (data not shown).

Receptor autoradiography

The relative proportion of the total [^{125}I]galanin binding
30 attributable to the GALR2 receptor was determined as the binding which was removed by 60 nM [D-Trp^2]galanin$_{(1-29)}$. The numerical representations in Table 2 indicate: 1) the relative intensity of the total binding obtained with [^{125}I]galanin, with +3 being the maximum; and 2) the
35 relative amount of this binding attributable to GALR2, with +3 again being the maximum.

Total [^{125}I]galanin binding was observed in many regions
of the rat brain, and was especially intense in the
forebrain, including the amygdala, parts of the
hypothalamus and thalamus, the septum, and the ventral
hippocampus. Other regions with intense binding signals
included the superior colliculus, the central gray, and
the dorsal horn of the spinal cord. The inclusion of 5
µM porcine galanin in the incubation resulted in a
complete displacement of [^{125}I]galanin binding from the rat
brain tissue sections. The use of 60 nM [D-Trp^2]galanin$_{(1-29)}$ partially displaced [^{125}I]galanin binding from many
regions of the rat brain.

The areas most affected by the GALR2 selective ligand
were the lateral septum, the paraventricular hypothalamic
nucleus, the centromedial and centrolateral thalamic
nuclei, the amygdalopiriform area of the amygdala, and
the superior colliculus. Other forebrain regions with
lesser but still significant reductions in [^{125}I]galanin
binding included the piriform and entorhinal cortices,
the globus pallidus, the supraoptic, lateral, and
ventromedial hypothalamic nuclei, and the anterior,
cortical, medial, and central amygdaloid nuclei. In the
midbrain, pons and medulla, [D-Trp^2]galanin$_{(1-29)}$ partially
reduced the total binding in the central gray, the raphe
obscurus and raphe magnus, the parabrachial nucleus, the
pontine reticular formation, the hypoglossal nucleus, and
the gigantocellular reticular nucleus.

In contrast, there were a number of areas in which [D-Trp^2]galanin$_{(1-29)}$ had little or no effect on the total
[^{125}I]galanin binding. Of these, the most striking were
the nucleus of the lateral olfactory tract, the ventral
hippocampus, and the dorsal horn of the spinal cord.
Other areas in which significant binding remained
included the olfactory bulb, the insular cortex, the
islands of Calleja, the nucleus accumbens, the lateral

- 106 -

habenula, the arcuate nucleus, and the spinal trigeminal
nucleus.

Developmental in situ hybridization

Using oligonucleotide probes, GalR2 mRNA appeared to be
developmentally regulated.   At P1 and P5, film
autoradiography of the hybridized brain sections revealed
clear signals over many thalamic nuclei.   In the
hypothalamus, both the paraventricular and ventromedial
nuclei were labeled. In addition, the superficial layers
of neocortex contained visible hybridization signal, as
did the dorsal hippocampus. In the mesencephalon, a low
level of hybridization signal was observed in the
pretectal region.

Ribonuclease protection assay

RNA was isolated and assayed as described from: heart,
striated muscle, liver, kidney, and CNS regions.   CNS
regions included: spinal cord, amygdala, hypothalamus,
cerebral cortex, cerebellum, and hippocampus.   The
highest levels of rGalR2 were detected in the
hypothalamus (Figure 14). Lower amounts were found in
heart, kidney, hippocampus amygdala, spinal cord, and
cerebellum (Figure 14). mRNA coding for the rGalR2 was
not detected in RNA extracted from striated muscle or
liver.

Generation of Human GALR2 PCR product:

Using PCR primers designed against the fourth and sixth
transmembrane domains of the rat GALR2 sequence, NS 525
and NS526, a 300 base pair fragment was amplified from 3
different lots of human genomic DNA. Sequence from all
three human genomic DNAs were >98% identical and
displayed 84% nucleotide identity to the rat GALR2 gene,
between the second extracellular domain and the 5' end of
the sixth transmembrane. This level of homology is
typical of a species homologue relationship in the GPCR

superfamily.

5' and 3' RACE Analysis of Human GALR2

5' RACE was performed on human brain RNA to isolate hGALR2 sequence upstream of the genomic PCR product above. Using nested reverse primers from the fifth transmembrane domain of hGALR2, a 600 base pair fragment was amplified. The sequence of this RACE product displayed 91% nucleotide identity to rGALR2 from the 3' end of the second transmembrane domain to the 5' end of the fifth transmembrane domain.

3' RACE was performed on human lung RNA to determine the sequence of the COOH terminus of hGALR2. Using nested forward primers from the fifth transmembrane domain of hGlR2, a 500 bp RACE product was generated that showed a 77% identity to nucleotides 1080-1139 of rGALR2. The sequence of this RACE product downstream from this region showed less homology to rGALR2, and was presumed to represent the COOH terminus and 3' UT of the hGALR2 gene.

Construction and Screening of a Human Heart cDNA Library

To obtain a full-length hGALR2 clone, superpools of a human heart cDNA library were screened by PCR using primers BB153 and BB169. A 325 base pair fragment was amplified from superpools 6, 9 and 16. Two positive primary pools, 69 and 72, were identified from superpool 9, and 1 positive primary pool, 121, was identified from superpool 16. One positive primary pool, 69, was subdivided into 48 pools of 3333 individual clones and screened by PCR. Twelve positive subpools were identified and one, 69-11, was subdivided into 20 pools of 1200 clones, plated onto agar plates, and screened by southern analysis. Thirty colonies that appeared positive were rescreened by PCR using primers BB167 and BB170, revealing 4 positive colonies. One of these, 69-11-5 was chosen for further analysis. To evaluate

- 108 -

whether this colony represented a single clone, a dilution of the colony was amplified on agar plates and colonies were screened by PCR using primers BB167 and BB170. Five of 20 colonies were positive for hGALR2, indicating that 69-11-5 was a mixture of 2 or more clones. One positive colony, 69-11-5-3, designated BO29, was amplified as a single hGlR2 clone. Vector-anchored PCR revealed that BO29 is in the correct orientation for expression, and encodes approximately 200 base pairs of 5'UT and 5000 base pairs 3'UT. Preliminary single-stranded sequence analysis indicates that BO29 encodes an initiating methionine and a termination codon, and contains an intron between the third and fourth transmembrane domains which is approximately 1.2kb in length. 69-11-5 has been demonstrated to confer ^{125}I galanin binding in transfected COS-7 cells, as assessed by microscopic analysis of photoemulsion-dipped slides. In addition, COS-7 cells transfected with the single clone BO29 exhibit significant binding of ^{125}I galanin in comparison with COS-7 cells transfected with control vector. In preliminary radioligand binding experiments, ^{125}I porcine galanin bound to membranes from COS-7 cells transfected with BO29, with a specific binding of 4900 fmol/mg, when the membranes (0.005 mg/ml) were incubated with 0.4 nM porcine galanin for 30 min. at 30° C. No specific binding was detected to membranes from mock-transfected COS-7 cells when tested under the same conditions.

Human GALR2 receptor pharmacology

A human GALR2 receptor construct containing an intron in the coding region of the cDNA (BO29) was prepared and transiently transfected into COS-7 cells. Human GALR2 receptors expressed in the COS-7 cell membranes were labeled by porcine ^{125}I-galanin with an apparent B_{max} of 4200 fmol/mg membrane protein and a K_d of 0.97 nM. The peptide binding profile for the human GALR2 receptor

(Table 8) resembled that reported previously for the rat
GALR2 in COS-7 cell membranes   (Table 1).

5   A human GALR2 receptor cDNA construct lacking the intron
in the coding region was also prepared (B039) and
transiently transfected into COS-7 cells. In a
preliminary experiment, membranes from transiently
transfected cells (membrane protein concentration = 0.045
mg/ml) were incubated with porcine ^{125}I-galanin (0.17 nM),
10   and specific binding was measured as 480 fmol/mg membrane
protein. Assuming an estimated K_d of 1 nM, the estimated
B_{max} for this construct would be ~3400 fmol/mg membrane
protein. Therefore, it is concluded that the absence of
the intron in the coding region of the human GALR2 cDNA
15   has no significant effect on receptor expression or
porcine ^{125}I-galanin binding.

Experimental Discussion

In order to clone additional members of the galanin
20   receptor family, an expression cloning strategy based on
the potential presence of multiple galanin receptors in
the hypothalamus was designed. Using this strategy a
cDNA clone encoding a galanin receptor from rat
hypothalamus, termed GALR2, was isolated that is distinct
25   from the previously cloned GALR1 receptors.

Transient transfection of the isolated cDNA (K985)
encoding GALR2 resulted in high affinity binding of [^{125}I]-
porcine galanin. The high binding affinity of the GALR2
30   receptor for galanin$_{(1-29)}$ and its truncated analogs
galanin$_{(1-16)}$ and galanin$_{(1-15)}$ strongly supports the notion
that the GALR2 receptor is a novel galanin receptor
subtype. Both the rat GALR1 and GALR2 receptors seem to
bind preferentially to the amino terminus of galanin.
35   Deletion of 13 or 14 amino acids from the carboxyl
terminus of galanin still yields peptides with high
binding affinity at both the GALR1 and GALR2 receptors.

- 110 -

Furthermore, the truncation of the first two amino acids of the amino terminus led to a complete loss of affinity at both GALR1 and GALR2. Consistent with this notion are the findings that the chimeric peptides, which share

5   identical amino acid sequences in the first 12 amino acids with galanin had very similar binding affinities for either GALR1 or GALR2 receptors. In spite of these similarities, the substitution of L-tryptophan with D-tryptophan in position 2 of porcine galanin$_{(1-29)}$ ([D-

10 Trp^2]galanin$_{(1-29)}$) led to a 7,000-fold loss in affinity at the GALR1 receptor compared to only a 14-fold reduction at the GALR2 receptor. The same substitution in the truncated analog galanin$_{(1-16)}$ led to a 4,200-fold reduction in affinity at the GALR1 receptor, and only a 6-fold

15 reduction in affinity at the GALR2 receptor. These data suggest that galanin analogs, with modifications at the 2-position, are better tolerated at the GALR2 receptor than at the GALR1 receptor as long as the side chain is an aromatic moiety.

20

Conversely, the substitution of tyrosine with alanine in position 9 of galanin$_{(1-16)}$, (i.e., to make [Ala]^9 galanin) leads to a 680-fold reduction in affinity at the GALR1 receptor and to a 60,000-fold reduction in affinity at

25 the GALR2 receptor. Altogether, the major differences in binding selectivity of the substituted analogs of galanin suggest the existence of substantial differences in the binding domains of these two receptor subtypes.

30 The existence of such structural differences between the GALR1 and GALR2 receptors are indicative of the potential for the design and discovery of novel subtype selective compounds. In this regard, the expression of the cDNA encoding the rat GALR2 receptors in cultured cell lines

35 provides a unique tool for the discovery of therapeutic agents targeted at galanin receptors.

- 111 -

Localization of galanin receptors

The high affinity of [D-Trp^2]galanin$_{(1-29)}$ for the cloned
GALR2 receptor (6 nM), and its low affinity for the GALR1
receptor (3 μM), makes it a useful tool for receptor
autoradiographic studies. Thus, brain areas in which the
total [^{125}I]galanin binding is significantly reduced by [D-
Trp^2]galanin$_{(1-29)}$ are interpreted as areas containing a
high proportion of GALR2 receptors, or other galanin
receptors with similar high affinity for [D-Trp^2]galanin$_{(1-29)}$. Those with lesser reductions are seen as regions
containing a higher concentration of GALR1 receptors.
The lateral septum, the paraventricular hypothalamic
nucleus, the centromedial and centrolateral thalamic
nuclei, the amygdalopiriform area of the amygdala, and
the superior colliculus all appear to contain primarily
GALR2 receptors. In contrast, the nucleus of the lateral
olfactory tract, the ventral hippocampus, and the dorsal
horn of the spinal cord appear to contain primarily GALR1
receptors. The predominance of the GALR1 receptor in
these regions is consistent with published reports of the
GALR1 messenger RNA localization (Parker et al., 1995;
Gustafson et al., 1996). In most other regions, there
appears to be a significant overlap between the two
subtypes.

While the functional implications of the GALR2 receptor
localization are not well understood at present, there
are a number of physiological processes attributable to
galanin that could be mediated by this receptor. These
include feeding (paraventricular hypothalamic nucleus),
cognition (septum and hippocampus), analgesia and/or
sensory processing (midline thalamic nuclei), and anxiety
and depression (amygdala and hypothalamus).

The observation that galanin is co-released with
norepinephrine from sympathetic nerve terminals suggests
that galanin could act via galanin receptors in the

periphery to modulate nearly every physiological process controlled by sympathetic innervation. Additional therapeutic indications not directly related to localization (supra) include diabetes, hypertension,
5   cardiovascular disorders, regulation of growth hormone release, regulation of fertility, gastric ulcers, gastrointestinal motility/transit/absorption/secretion, glaucoma, inflammation, immune disorders, respiratory disorders (eg. asthma, emphysema).
10

The physiological and anatomical distribution of galanin-containing neurons suggests potential roles of galanin receptors mediating effects on cognition, analgesia, neuroendocrine regulation, control of insulin release and
15   control of feeding behavior. Of particular relevance to the role of the novel GALR2 receptor, are those functions mediated by galanin receptors in the rat hypothalamus.

Studies in rats indicate that the injection of galanin in
20   the hypothalamus increases food intake (Kyrouli et al, 1990, and Schick et al, 1993) and that this stimulatory effect of galanin is blocked by prior administration of M40 and C7 (Liebowitz and Kim, 1992; and Corwin, 1993). The expression of the mRNA encoding the GALR1 receptor in
25   the rat hypothalamus, (Parker et al., 1995, Gustafson et al., 1996) and the fact that the novel GALR2 receptor was cloned from a cDNA library prepared from rat hypothalamus argues in favor of either receptor subtype to be involved in the regulation of feeding behavior (Parker et al.,
30   1996). However, the evidence against the involvement of GALR1 in the stimulation of feeding behavior stems from the fact that M40 and C7 are known to be agonists, and not antagonists, in cell lines expressing the cloned human and rat GALR1 receptors (Heuillet et al. 1994; Hale
35   et al. 1993; and Bartfai et al. 1993).

The distribution of GALR2 mRNA in the rat brain and

- 113 -

periphery has been determined by ribonuclease protection
assay, in situ hybridization, Northen blot analysis and
RT-PCR. The results of these studies suggest that this
receptor is potentially involved in mediating many of the
5   physiological roles ascribed to the peptide galanin. In
the adult rat, localization of the GalR2 mRNA in the
hypothalamus indicates a role for this receptor in
homeostatic mechanisms, including food intake and
neuroendocrine regulation. The presence of GALR2 mRNA in
10   the neocortex and dorsal hippocampus suggest an
involvement in cognition, which is consistent with
documented changes in galanin and galanin receptor
expression during aging and in the brains of Alzheimer's
patients (Chan-Palay, 1988; Leverenz et al., 1996).
15   Galanin also has antinociceptive effects, and the
localization of GALR2 mRNA in the spinal cord (present
investigation) and dorsal root ganglia (O'Donnell et al.,
1996) implicate this receptor in pain neurotransmission.
The localization of GALR2 mRNA in the cerebellum is
20   intriguing, as it suggests a role for galanin and the
GalR2 receptor in planned movements and potentially in
movement disorders.

In addition to the localization observed in adult
25   animals, it also appears that the GALR2 mRNA is
developmentally regulated, with the highest levels
observed early in postnatal development. Thus, it is
possible that this galanin receptor plays a role in
developmental processes which occur during the first
30   postnatal week, such as axonal guidance and synapse
formation.

A unique pharmacological profile for the GALR2 receptor
has been generated through binding and functional assays.
35   This profile can be used to deduce the physiological
function of the GalR2 receptor in vivo. Consider the
agonist activity of galanin 1-16, for example. Galanin

- 114 -

1-16 is reported to function as an agonist in various
models of hypothalamic, pituitary and pancreatic function
(Kask et al.)   Galanin 1-16 is also reported to mimic
the effects of galanin on the flexor reflex in the rat.
The N-terminally extended peptides galanin (-7) to (+) 29
and galanin (-9) to (+) 29, also characterized as rat
GALR2 agonists, can mimic the effects of galanin in a rat
flexor reflex assay (Weisenfeld). Taken together, these
data suggest a potential role for the rat GalR2 receptor
in a range of physiology or pathophysiology including
diabetes, pain, reproduction, obesity and eating
disorders.

The agonist activity of M40 in GALR2 in vitro assays is
particularly intriguing when viewed in the context of
behavioral feeding models. From the literature, one
might conclude that the agonist activity of M40 in vitro
is in apparent conflict with the antagonist activity
reported for M40 in behavioral models of food intake, and
that the GALR2 receptor is therefore unlikely to mediate
the feeding response. The data generated and reported in
the subject application do not support this conclusion.
Rather, the data from behavioral feeding models indicate
that M40 is an orexigenic peptide whose maximal effect
is comparable to that for galanin itself.   The agonist
activity reported herein for M40 both in vitro and in
vivo is consistent with the proposal that the rat GALR2
receptor mediates the stimulatory effect of galanin on
food intake in the central nervous system. These data
further suggest that the rat GALR2 receptor represents
a target for the design of therapeutic compounds for the
treatment of obesity and related disorders.

- 115 -

Table 1: Binding of galanin peptide analogs to the recombinant rat GALR1 and GALR2 receptors transiently expressed in COS 7 cells.

Analog	GALR1 (pKi)		GALR2 (pKi)	
	Mean	SEM*	Mean	SEM
porcine galanin (1-29)	9.34	0.15	9.35	0.14
[D-Trp²]porcine galanin (1-29)	5.46	0.04	8.19	0.26
[Phe²]porcine galanin (1-29)	5.99	0.13	5.64	0.11
[D-Ala⁷]porcine galanin (1-29)	8.66	0.04	8.76	0.09
galanin (1-16)	8.66	0.01	8.76	0.13
[D-Trp²]galanin (1-16)	4.40	0.09	8.02	0.10
[Ala⁵]galanin (1-16)	6.27	0.05	7.46	0.13
[Ala⁹]galanin (1-16)	5.83	0.02	3.98	0.10
galanin (1-15)	8.47	0.04	9.19	0.06
[Phe²]galanin (1-15)	4.63	0.03	5.85	0.49
porcine galanin (3-29)	< 4.0		< 4.0	
galantide	8.02	0.08	8.70	0.07
C-7	7.79	0.01	7.72	0.09
M32	9.21	0.10	9.23	0.05
M35	9.48	0.07	9.24	0.10
M40	8.44	0.09	9.14	0.21

*SEM=standard error of the mean, from 3 independent experiments.

- 116 -

Table 2. Distribution of [^{125}I]galanin binding in rat brain. Total binding is compared to the amount attributable to GALR2 (as indicated by displacement of [^{125}I]galanin by 60 nM [D-Trp^2]porcine galanin$_{(1-29)}$).

Region	Total [^{125}I]Gal binding	Putative GALR2 sites	Potential Applications
Olfactory bulb	+3	+1	Modulation of olfactory sensation
Anterior olfactory n.	+3	+1	Modulation of olfactory sensation
Cortex			
dorsal neocortex, layer 4	+1	+1	Sensory integration
piriform	+2	+1	Modulation of olfactory sensation
agranular insular	+3	+1	Processing of visceral information
entorhinal	+2	+1	
dorsal endopiriform	+2	+1	
Claustrum	+2	+1	Visual processing
Basal ganglia			
n. accumbens	+2	0	Modulation of dopaminergic function
olfactory tubercle	+2	+1	
globus pallidus	+1	+1	
islands of Calleja	+3	+1	
Septal area			Cognitive enhancement via cholinergic system
lateral septum	+3	+2	
diagonal band n.	+2	0	
Hypothalamus			
anterior	+1	0	Neuroendocrine regulation
supraoptic n.	+2	+1	
paraventricular	+2	+2	Appetite/obesity
ventromedial	+2	+1	

Region	Total [^{125}I]Gal binding	Putative GALR2 sites	Potential Applications
arcuate	+1	0	
lateral	+2	+1	
medial mammillary	+2	+1	
Thalamus			Analgesia/sensory modulation
paraventricular n.	+1	0	
centromedial	+3	+2	
paracentral	+3	+1	
rhomboid	+1	0	
reuniens	+2	+1	
mediodorsal	+2	0	
reticular n.	+1	+½	
centrolateral n.	+3	+2	
zona incerta	+2	+1	
lateral dorsal	+1	+½	
habenula	+3	+1	Anxiety/sleep disorders
Hippocampus			Cognition enhancement/ ischaemia
Ca1, ventral	+3	0	
subiculum	+2	+1	
Amygdala			Anxiolytic, appetite, depression
bed n. stria terminalis	+3	+1	
n. lateral olfactory tract	+3	0	
Amygdala			Anxiolytic, appetite, depression
anterior	+2	+1	
medial	+3	+1	
cortical	+2	+1	
central	+3	+1	
amygdalohippocampal	+2	0	
amygdalopiriform	+3	+2	
Midbrain			

Region	Total [^{125}I]Gal binding	Putative GALR2 sites	Potential Applications
superior colliculus	+3	+2	Visual function
raphe obscurus	+2	+1	Analgesia
central gray	+2	+1	Analgesia
Pons/medulla			
raphe magnus	+2	+1	Analgesia
parabrachial n.	+2	+1	
pontine ret. n.	+2	+1	
reticulotegmental	+2	+1	
gigantocellular	+2	+1	
motor trigeminal	+1	0	
spinal trigeminal	+3	+1	Migraine
hypoglossal n.	+2	+1	Motor coordination
area postrema	+1	0	
Spinal cord			
dorsal horn	+3	+1	Analgesia

Table 3. Northern blot hybridization of GALR2 receptor
in brain and various peripheral rat tissues.

Tissue	Blot 1	Blot 2	Mean Signal	Therapeutic Indications
Heart	+++	++	2.5	Cardiovascular Indications (including hypertension and heart failure)
Brain	++++	++++	4.0	Obesity/feeding, analgesia, cognition enhancement, Alzheimer's disease, depression, anxiety, sleep disorders, Parkinson's disease, traumatic brain injury, convulsion/epilepsy
Spleen	++	++	2.0	Immune functions, hematopoiesis
Lung	++++	++++	4.0	Respiratory disorders, asthma, emphysema, lung cancer diagnostics
Liver	++	-	1.0	Diabetes
Skeletal Muscle	+	++	1.5	Diabetes
Kidney	+++	+++	3.0	Hypertension, electrolyte balance, diuretic, anti-diuretic
Testis	+++	+	2.0	Reproductive function

Table 4.   Inositol phosphate hydrolysis in LM(tk-) cells
stably transfected with GALR2.

Peptide	EC_{50} PI (nM) rat GALR2 (with intron)	EC_{50} PI (nM) L-rGALR2I-4 (intronless)
porcine galanin	21	14
M35	29	28
D-Trp^2-galanin 1-16	1380	660
D-Trp^2-galanin 1-29	200	230
galanin 1-16	65	18
M40	28	47
M32	13	35

- 121 -

Table 5. Rat GALR2 receptors stably transfected in CHO
Comparison of binding data, phosphatidyl inositol
release, and arachidonic acid release in C-rGalR2-79.

Peptide	K_i from porcine ^{125}I-galanin binding assays (nM)	EC_{50} from PI hydrolysis assays (nM)	EC_{50} from arachidonic acid assays (nM)
rat galanin	0.52	14	0.67
porcine galanin	0.94	15	1.3
porcine galanin 1-16	3.5	91	2.6
D-Trp^2-galanin 1-16	110	590	50

- 122 -

Table 6. CHO GALR2 pharmacology: binding (K_i vs. ^{125}I-porcine galanin) vs. function (arachidonic acid hydrolysis)

Peptide	Rat GALR2 K_i, C-rGalR2-79 (nM)	Rat GALR2 EC_{50} AA, C-rGalR2-79 (nM)
Human galanin	1.2	
Porcine galanin	0.94	1.3
rat galanin	0.52	0.67
porcine gal -7 to + 29		3.0
porcine galanin -9 to + 29		4.0
porcine galanin 3-iodo-L-Tyr9-galnin		0.8
porcine galanin 3-iodo-L-Tyr26 galanin		1.0
porcine Phe2-galanin	> 1000	
porcine D-Trp2-galanin	41	11
D-Trp2-3-iodo-L-Tyr9-galanin		3.0
porcine D-Trp2-3-iodo-L-Tyr26-galanin		6.0
D-Ala7-galanin	6.2	5.4
porcine galanin 3-29	> 1000	> 1000
porcine galanin 9-29	> 1000	> 1000
porcine galanin 17-29	> 1000	
porcine galanin 1-16	3.5	2.6
porcine Ala2-galanin 1-16	> 1000	
porcine D-Trp2-galanin 1-16	110	50
porcine Ala5-galanin 1-16	> 620	
porcine Ala9-galanin 1-16	> 1000	
porcine galanin 1-15	1.5	2.3
Phe2-galanin 1-16	> 1000	> 1000
porcine galanin 1-12	2.1	2.3
porcine galanin 1-9	> 1000	> 1000

- 123 -

Peptide	Rat GALR2 K_i, C-rGalR2-79 (nM)	Rat GALR2 EC_{50} AA, C-rGalR2-79 (nM)
C7	48	2.4
Galantide	4.9	0.93
M32	3.4	2.5
M35	5.8	1.3
M40	3.5	2.7

Table 7. Peptide-dependent activation of rat GALR1 vs. rat GALR2.

Peptide	LM(tk-) Rat GALR1 cAMP assay EC_{50} (nM)	C-RGalR2-79 arachidonic acid assay EC_{50} (nM)
Porcine galanin	0.06	1.3
rat galanin	0.05	0.67
porcine D-Trp^2-galanin	> 850	11
porcine galanin 3-29	> 1000	> 1000
porcine galanin 1-16	0.34	2.6
porcine D-Trp^2-galanin 1-16	> 1000	50
C7	0.52	2.4
Galantide	0.08	0.93
M32	0.34	2.5
M35	0.15	1.3
M40	0.82	2.7

Table 8. Peptide binding profile: Human GALR2 vs. rat GALR2 transiently expressed in COS-7

Peptide	Human GALR2 K_i (nM)	Rat GALR2 K_i (nM)
porcine galanin 1-16	15	7.2*
porcine galanin	0.72	0.45
M40	5.3	0.72
porcine D-Trp^2-galanin	290	52*
M32	7.9	12*
rat galanin	1.0	0.52*

*additional experiments were performed with some of the peptides shown in Table 1.

- 126 -

References

Ahrén, B. and S. Lindskog (1992) Int. J. Pancreatol. 11:147-160.

5   Amiranoff, B. A.M. Lorinet, and M. Laburthe (1991) Eur. J. Biochem. 195:459-463.

Amiranoff, B. A.L. Servin, C. Rouyer-Fessard, A. Couvineau, K. Tatemoto, and M. Laburthe (1987) Endocrin.
10   121:284-289.

Aruffo, A. and B. Seed (1987) Proc. Natl. Acad. Sci. USA 84:8573-8577.

15   Bhathena, S.J., H.K. Oie, A.F. Gazdar, N.R.Voyles, S.D. Wilkins, and L. Recant (1982) Diabetes 31:521-531.

Bartfai,T., K. Bedecs, T. Land, Ü. Langel, R. Bertorelli, P. Girotti, S. Consolo, Y.-J. Yu, Z.
20   Weisenfeld-Hallin, S. Nilsson, V. Pieribone, and T. Hökfelt (1991) Proc. Natl. Acad. Sci. USA 88:10961-10965.

Bartfai,T., T. Hokfelt, and U. Langel, Crit. Rev.
25   Neurobiol. 7:229-274.

Bartfai,T., Ü. Langel, K. Bedecs, S. Andell, T. Land, S. Gregersen, B. Ahren, P. Girotti, S. Consolo, R. Corwin, J. Crawley, X. Xu, Z. Weisenfeld-Hallin, and T. Hökfelt
30   (1993) Proc. Natl. Acad. Sci. USA 88:11287-11291.

Bennet, W.M., S.F. Hill, M.A. Ghatei, and S.R. Bloom (1991) J. Endocrin. 130:463-467.

35   Borden, L.A., K.E. Smith., P.R. Hartig, T.A. Branchek, and R.L. Weinshank (1992) J. Biol. Chem. 267: 21098-21104.

Borden, L.A., K.E. Smith, E.L. Gustafson, T.A. Branchek, and R.L. Weinshank (1995) J. Neurochem. 64977-984.

5   Boyle, M.R., C. B. Verchere, G. McKnight, S. Mathews, K. Walker, and G.J. Taborsky, Jr. (1994) Reg. Peptides 50:1-11.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein 10   utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Burbach, J.P. and O.C. Meijer (1992) Eur. J. Pharmacol. 227:1-18.
15
Burgevin, M.-C., Loquet, I., Quarteronet, D., and Habert-Ortoli, E. (1995) J. Molec. Neurosci., 6:33-41.

Burns, C.M., Chu, H., Rueter, S.M., Sanders-Bush, E., and 20   R.B. Erneson. (1996) Neuroscience Abstracts 385.9.

Bush, A.W., Borden, L.A., Greene, L.A., and Maxfield, F.R. (1991) J. Neurochem. 57:562-574.

25   Chan-Palay, V. (1988) J.Comp.Neurol. 273:543-557.
Chen, Y., A. Fournier, A. Couvineau, M. Laburthe, and B. Amiranoff (1993) Proc. Natl. Acad. Sci. USA 90:3845-3849.

30   Chirgwin, J.M., A.E. Przybyla, R.J. MacDonald, and W.J. Rutter. (1979) Biochemistry 18:5294-5299.

Chu, H., Burns, C., Canton, H., Emeson, R.B., and E. Sanders-Bush. (1996) Neuroscience Abstracts 385.10.
35
Consolo, S., R. Bertorelli, P. Girotti, C. La Porta, T. Bartfai, M. Parenti, and M. Zambelli (1991) Neurosci.

Lett. 126:29-32.

Crawley, J.N. (1993) Behav. Brain Res. 57:133-141.

Crawley, J.N., J.K. Robinson, Ü. Langel, and T. Bartfai (1993) Brain. Res. 600:268-272.

Cullen, B. (1987). Use of eurkaryotic expression technology in the functional analysis of cloned genes. Methods Enzymol. 152: 685-704.

D'Andrea, A.D., H.F. Lodish, and G.W. Gordon (1989) Cell 57:277-285.

Fisone, G., C.F. Wu, S. Consolo, Ö. Nördstrom, N. Brynne, T. Bartfai, T. Melander, T. Hökfelt (1987) Proc. Natl. Acad. Sci USA 84:7339.

Gearing, D.P., King, J.A.,Gough, N.M. and Nicola N.A. (1989) EMBO J. 8:3667-3676.

Gerald, C., M. Walker, T. Branchek, and R. Weinshank (1994) DNA Encoding a Human Neuropeptide Y/Peptide YY (Y2) Receptor and Uses Thereof, United States Patent Application US Serial No. 08/192,288, filed February 3, 1994.

Gillison, S.L., and W.G. Sharp (1994) Diabetes 43:24-32. Gregersen, S., S. Lindskog, T. Land, U. Langel, T. Bartfai, and B. Ahren (1993) Eur J. Pharmacol. 232:35-39.

Gu, Z.-F., W.J. Rossowski, D.H. Coy, T.K. Pradhan, and R.T. Jensen (1993) J. Phamacol. Exper. Ther. 266:912-918.

Gu, Z.-F., Pradhan, T.K., Coy, D.H., and Jensen, R.T. (1995) J. Pharmacol. Exp. Ther., 272:371-378.

- 129 -

Gubler, U abd B.J. Hoffman. (1983). A simple and very efficient method for generating cDNA libraries. Gene. 25, 263-269

5   Gundersen, C. B., R. Miledi, and I. Parker. 1983. *Proc. R. Soc. London Ser.* B 219:103-109.

Gustafson, E.L., Smith, K.E., Durkin, M.M., Gerald, C., and Branchek, T.A. (1996) Neuroreport, 7:953-957.

10

Habert-Ortoli, E., Amiranoff, B., Loquet, I., Laburthe, M., and J.-F. Mayaux (1994) Proc. Natl. Acad. Sci.USA 91:9780-9783.

15   Hedlund, P.B., N. Yanaihara, and K. Fuxe (1992) Eur. J. Pharm. 224:203-205.

Heuillet, E., Bouaiche, Z., Menager, J., Dugay, P., Munoz, N., Dubois, H., Amiranoff, B., Crespo, A.,
20   Lavayre, J., Blanchard, J.-C., and Doble, A. (1994) Eur. J. Pharmacol., 269:139-147.

Kaplan, L.M., S.M. Gabriel, J.I. Koenig, M.E. Sunday, E.R. Spindel, J.B. Martin, and W.W. Chin (1988) Proc.
25   Natl. Acad. Sci.USA 85:7408-7412.

Kieffer, B., Befort, K., Gaveriaux-Ruff, C. and Hirth, C.G. (1992). The δ-opioid receptor: Isolation of a cDNA by expression cloning and pharmacological
30   characterization. Proc. Natl. Acad. Sci. USA 89:12048-12052.

Kluxen, F.W., Bruns, C. and Lubbert H. (1992). Expression cloning of a rat brain somatostatin receptor cDNA. Proc.
35   Natl. Acad. Sci. USA 89:4618-4622.

Kornfeld, R. and Kornfeld, S. (1985). Assembly of

- 130 -

asparagine linked oligosaccharides. _Annu. Rev. Biochem_.
54:631-664.

Kozak, M. (1989). The scanning model for translation: an
update. _J. Cell Biol._ 108: 229-241.

Kozak, M. (1991). Structural features in eukaryotic
mRNAs that modulate the initiation of translation. _J.
Biol. Chem_. 266: 19867-19870.

Kyrkouli, S.E., B.G. Stanley, R.D. Seirafi and S.F.
Leibowitz (1990) _Peptides_ 11:995-1001.

Lagny-Pourmir, I., A.M. Lorinet, N. Yanaihara, and M.
Laburthe (1989) _Peptides_ 10:757-761.

Landschultz, W.H., Johnson, P.F. and S.L. McKnight.
(1988). The leucine zipper: a hypothetical structure
common to a new class of DNA binding proteins. _Science_
240: 1759-1764.

Leibowitz, S.F. and T. Kim (1992) _Brain Res._ 599:148-152.

Maggio, R., Vogel Z. and J. Wess. (1993). Coexpression
studies with mutant muscarinic/adrenergic receptors
provide evidence for intermolecular "cross-talk" between
G-protein-linked receptors. _Proc. Natl. Acad. Sci. USA_
90: 3103-3107.

McCormick, M. (1987). Sib Selection. _Methods in
Enzymology_, 151: 445-449.

Melander, T., C. Köhler, S. Nilsson, T. Hökfelt, E.
Brodin, E. Theodorsson, and T. Bartfai (1988) _J. Chem.
Neuroanat._ 1:213-233.

Merchenthaler, I., F.J. López, and A. Negro-Vilar (1993)

- 131 -

Prog. Neurobiol. 40:711-769.

Miller, J. and Germain, R.N. (1986). Efficient cell surface expression of class II MHC molecules in the absence of associated invariant chain. J. Exp. Med. 164: 1478-1489.

Ögren, S.-O., T. Hökfelt, K. Kask, Ü. Langel, and T. Bartfai (1992) Neurosci. 51:1.

Palazzi, E., G. Fisone, T. Hökfelt, T. Bartfai, and S. Consolo (1988) Eur. J. Pharmacol. 148:479.

Parker, E.M., Izzarelli, D., Nowak, H., Mahle, C., Iben, L., Wang, J., and Goldstein, M.E. (1995) Mol. Brain Res., 34:179-189.

Post, C., L. Alari, and T. Hökfelt (1988) Acta Physiol. Scand. 132:583.

Probst, W.C., Snyder, L.A., Schuster, D.I., Brosius, J and Sealfon, S.C. (1992). Sequence alignment of the G-protein coupled receptor superfamily. DNA and Cell Bio. 11: 1-20.

Quick, M. W. and H. A. Lester. 1994. Methods for expression of excitability proteins in Xenopus oocytes. Meth. Neurosci. 19:261-279.

Sanger, S. (1977) Proc. Natl. Acad. Sci. USA 74:5463-5467.

Servin, A.L., B. Amiranoff, C. Rouyer-Fessard, K. Tatemoto, and M. Laburthe (1987) Biochem. Biophys. Res. Comm. 144:298-306.

Shen, Y., Monsma, F.J. Jr., Metcalf, M.A., Jose, P.A.,

- 132 -

Hamblin, M.W., and Sibley, D.R. (1993) Molecular Cloning and Expression of a 5-Hydroxytryptamine₇ Serotonin Receptor Subtype. J. Biol. Chem. 268:18200-18204.

5   Sims, J.E., C.J. March, D. Cosman, M.B. Widmer, H.R. Macdonald, C.J. McMahan, C.E. Grubin, J.M. Wignal, J.L. Jackson, S.M. Call, D. Freind, A.R. Alpert, S. Gillis, D.L. Urdal, and S.K. Dower (1988) Science 241:585-588.

10   Skofitsch, G. and D.M. Jacobowitz (1985) Peptides 6:509-546.

Skofitsch, G., M.A. Sills, and D.M. Jacobowitz (1986) Peptides 7:1029-1042.

15

Smith, K.E., L.A. Borden, P.R. Hartig, T. Branchek, and R.L. Weinshank (1992) Neuron 8: 927-935.

Smith, K.E., L.A. Borden, C-H.D. Wang, P.R. Hartig, T.A.
20   Branchek, and R.L. Weinshank (1992a) Mol. Pharmacol. 42:563-569.

Smith, K.E., S.G. Fried, M.M. Durkin, E.L. Gustafson, L.A. Borden, T.A. Branchek, and R.L. Weinshank (1995)
25   FEBS Letters, 357:86-92.

Sundström, E., T. Archer, T. Melander, and T. Hökfelt (1988) Neurosci. Lett. 88:331.

30   Takahashi, T., E. Neher, and B. Sakmann. 1987. Rat brain serotonin receptors in Xenopus oocytes are coupled by intracellular calcium to endogenous channels. Proc. Natl. Acad. Sci. USA 84:5063-6067.

35   Tempel, D.L., K.J. Leibowitz, and S.F. Leibowitz (1988) Peptides 9:300-314.

Vrontakis, M.E., L.M. Peden, M.L Duckworth, and H.G.
Friesen (1987) J. Biol. Chem. 262:16755-16760.

Warden, D. and H.V. Thorne. (1968). Infectivity of
polyoma virus DNA for mouse embryo cells in presence of
diethylaminoethyl-dextran. J. Gen. Virol. 3: 371.

Wiesenfeld-Hallin, Z., X.J. Xu, J.X. Hao, and T. Hökfelt
(1993) Acta Physiol.Scand. 147:457-458.

Wiesenfeld-Hallin, Z., et al. (1992) Proc. Natl. Acad.
Sci. USA 89:3334-3337.

Wynick D., D.M. Smith, M. Ghatei, K. Akinsanya, R.
Bhogal, P. Purkiss, P. Byfield, N. Yanaihara, and S.R.
Bloom (1993) Proc. Natl. Acad. Sci. USA 90:4231-4245.0

SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT: Smith, Kelli E.
                Gerald, Christophe P.G.
                Weinshank, Richard L.
                Linemeyer, David
                Branchek, Theresa
                Forray, Carlos

    (ii) TITLE OF INVENTION: DNA ENCODING GALANIN GALR2 RECEPTORS
                      AND USES THEREOF

    (iii) NUMBER OF SEQUENCES: 32

    (iv) CORRESPONDENCE ADDRESS:
        (A) ADDRESSEE: Cooper & Dunham LLP
        (B) STREET: 1185 Avenue of the Americas
        (C) CITY: New York
        (D) STATE: New York
        (E) COUNTRY: U.S.A.
        (F) ZIP: 10036

    (v) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

    (vi) CURRENT APPLICATION DATA:
        (A) APPLICATION NUMBER:
        (B) FILING DATE:
        (C) CLASSIFICATION:

    (viii) ATTORNEY/AGENT INFORMATION:
        (A) NAME: White, John P.
        (B) REGISTRATION NUMBER: 28,678
        (C) REFERENCE/DOCKET NUMBER: 50233-D-PCT/JPW/MAT

    (ix) TELECOMMUNICATION INFORMATION:

- 135 -

                    (A) TELEPHONE: 212-278-0400
                    (B) TELEFAX: 212-391-0525

    (2) INFORMATION FOR SEQ ID NO:1:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 24 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGGGCAACAG CCTAGTGATC ACCG   24

    (2) INFORMATION FOR SEQ ID NO:2:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 24 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CTGCTCCCAG CAGAAGGTCT GGTT   24

    (2) INFORMATION FOR SEQ ID NO:3:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 24 base pairs
            (B) TYPE: nucleic acid

- 136 -

          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: DNA

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCTCAGTGAA GGGAATGGGA GCGA   24

(2) INFORMATION FOR SEQ ID NO:4:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 26 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: DNA

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTCATTGCAA ACACGGCACT TGAACA   26

(2) INFORMATION FOR SEQ ID NO:5:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 24 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: DNA

                                 - 137 -

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTTGCTTGTA CGCCTTCCGG AAGT   24

(2) INFORMATION FOR SEQ ID NO:6:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 24 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GAGAACTTCA TCACGCTGGT GGTG   24

(2) INFORMATION FOR SEQ ID NO:7:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 1119 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (ix) FEATURE:
            (A) NAME/KEY: CDS
            (B) LOCATION: 1..1119

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATG AAT GGC TCC GGC AGC CAG GGC GCG GAG AAC ACG AGC CAG GAA GGC   48
Met Asn Gly Ser Gly Ser Gln Gly Ala Glu Asn Thr Ser Gln Glu Gly
 1   5   10   15

- 138 -

```
GGT AGC GGC GGC TGG CAG CCT GAG GCG GTC CTT GTA CCC CTA TTT TTC  96
Gly Ser Gly Gly Trp Gln Pro Glu Ala Val Leu Val Pro Leu Phe Phe
            20              25              30


GCG CTC ATC TTC CTC GTG GGC ACC GTG GGC AAC GCG CTG GTG CTG GCG  144
Ala Leu Ile Phe Leu Val Gly Thr Val Gly Asn Ala Leu Val Leu Ala
            35              40              45


GTG CTG CTG CGC GGC GGC CAG GCG GTC AGC ACC ACC AAC CTG TTC ATC  192
Val Leu Leu Arg Gly Gly Gln Ala Val Ser Thr Thr Asn Leu Phe Ile
        50              55              60


CTC AAC CTG GGC GTG GCC GAC CTG TGT TTC ATC CTG TGC TGC GTG CCT  240
Leu Asn Leu Gly Val Ala Asp Leu Cys Phe Ile Leu Cys Cys Val Pro
    65              70              75              80


TTC CAG GCC ACC ATC TAC ACC CTG GAC GAC TGG GTG TTC GGC TCG CTG  288
Phe Gln Ala Thr Ile Tyr Thr Leu Asp Asp Trp Val Phe Gly Ser Leu
                85              90              95


CTC TGC AAG GCT GTT CAT TTC CTC ATC TTT CTC ACT ATG CAC GCC AGC  336
Leu Cys Lys Ala Val His Phe Leu Ile Phe Leu Thr Met His Ala Ser
                100             105             110


AGC TTC ACG CTG GCC GCC GTC TCC CTG GAC AGG TAT CTG GCC ATC CGC  384
Ser Phe Thr Leu Ala Ala Val Ser Leu Asp Arg Tyr Leu Ala Ile Arg
            115             120             125


TAC CCG CTG CAC TCC CGA GAG TTG CGC ACA CCT CGA AAC GCG CTG GCC  432
Tyr Pro Leu His Ser Arg Glu Leu Arg Thr Pro Arg Asn Ala Leu Ala
        130             135             140


GCC ATC GGG CTC ATC TGG GGG CTA GCA CTG CTC TTC TCC GGG CCC TAC  480
Ala Ile Gly Leu Ile Trp Gly Leu Ala Leu Leu Phe Ser Gly Pro Tyr
145             150             155             160


CTG AGC TAC TAC CGT CAG TCG CAG CTG GCC AAC CTG ACA GTA TGC CAC  528
Leu Ser Tyr Tyr Arg Gln Ser Gln Leu Ala Asn Leu Thr Val Cys His
                165             170             175


CCA GCA TGG AGC GCA CCT CGA CGT CGA GCC ATG GAC CTC TGC ACC TTC  576
Pro Ala Trp Ser Ala Pro Arg Arg Arg Ala Met Asp Leu Cys Thr Phe
```

```
                 180                    185                    190

GTC TTT AGC TAC CTG CTG CCA GTG CTA GTC CTC AGT CTG ACC TAT GCG 624
Val Phe Ser Tyr Leu Leu Pro Val Leu Val Leu Ser Leu Thr Tyr Ala
        195                    200                    205


CGT ACC CTG CGC TAC CTC TGG CGC ACA GTC GAC CCG GTG ACT GCA GGC 672
Arg Thr Leu Arg Tyr Leu Trp Arg Thr Val Asp Pro Val Thr Ala Gly
        210                    215                    220


TCA GGT TCC CAG CGC GCC AAA CGC AAG GTG ACA CGG ATG ATC ATC ATC 720
Ser Gly Ser Gln Arg Ala Lys Arg Lys Val Thr Arg Met Ile Ile Ile
225                    230                    235                    240


GTG GCG GTG CTT TTC TGC CTC TGT TGG ATG CCC CAC CAC GCG CTT ATC 768
Val Ala Val Leu Phe Cys Leu Cys Trp Met Pro His His Ala Leu Ile
                245                    250                    255


CTC TGC GTG TGG TTT GGT CGC TTC CCG CTC ACG CGT GCC ACT TAC GCG 816
Leu Cys Val Trp Phe Gly Arg Phe Pro Leu Thr Arg Ala Thr Tyr Ala
                260                    265                    270


TTG CGC ATC CTT TCA CAC CTA GTT TCC TAT GCC AAC TCC TGT GTC AAC 864
Leu Arg Ile Leu Ser His Leu Val Ser Tyr Ala Asn Ser Cys Val Asn
                275                    280                    285


CCC ATC GTT TAC GCT CTG GTC TCC AAG CAT TTC CGT AAA GGT TTC CGC 912
Pro Ile Val Tyr Ala Leu Val Ser Lys His Phe Arg Lys Gly Phe Arg
        290                    295                    300


AAA ATC TGC GCG GGC CTG CTG CGC CCT GCC CCG AGG CGA GCT TCG GGC 960
Lys Ile Cys Ala Gly Leu Leu Arg Pro Ala Pro Arg Arg Ala Ser Gly
305                    310                    315                    320
```

```
CGA GTG AGC ATC CTG GCG CCT GGG AAC CAT AGT GGC AGC ATG CTG GAA 1008
Arg Val Ser Ile Leu Ala Pro Gly Asn His Ser Gly Ser Met Leu Glu
            325             330             335


CAG GAA TCC ACA GAC CTG ACA CAG GTG AGC GAG GCA GCC GGG CCC CTT 1056
Gln Glu Ser Thr Asp Leu Thr Gln Val Ser Glu Ala Ala Gly Pro Leu
            340             345             350


GTC CCA CCA CCC GCA CTT CCC AAC TGC ACA GCC TCG AGT AGA ACC CTG 1104
Val Pro Pro Pro Ala Leu Pro Asn Cys Thr Ala Ser Ser Arg Thr Leu
            355             360             365


GAT CCG GCT TGT TAA                                              1119
Asp Pro Ala Cys  *
            370
```

(2) INFORMATION FOR SEQ ID NO:8:

         (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 373 amino acids
             (B) TYPE: amino acid
             (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Asn Gly Ser Gly Ser Gln Gly Ala Glu Asn Thr Ser Gln Glu Gly
 1               5              10              15


Gly Ser Gly Gly Trp Gln Pro Glu Ala Val Leu Val Pro Leu Phe Phe
            20              25              30


Ala Leu Ile Phe Leu Val Gly Thr Val Gly Asn Ala Leu Val Leu Ala
            35              40              45


Val Leu Leu Arg Gly Gly Gln Ala Val Ser Thr Thr Asn Leu Phe Ile
    50              55              60


Leu Asn Leu Gly Val Ala Asp Leu Cys Phe Ile Leu Cys Cys Val Pro
65              70              75              80
```

- 141 -

```
Phe Gln Ala Thr Ile Tyr Thr Leu Asp Asp Trp Val Phe Gly Ser Leu
                  85              90                  95


Leu Cys Lys Ala Val His Phe Leu Ile Phe Leu Thr Met His Ala Ser
              100             105             110


Ser Phe Thr Leu Ala Ala Val Ser Leu Asp Arg Tyr Leu Ala Ile Arg
          115             120             125


Tyr Pro Leu His Ser Arg Glu Leu Arg Thr Pro Arg Asn Ala Leu Ala
    130             135             140


Ala Ile Gly Leu Ile Trp Gly Leu Ala Leu Leu Phe Ser Gly Pro Tyr
145             150             155             160


Leu Ser Tyr Tyr Arg Gln Ser Gln Leu Ala Asn Leu Thr Val Cys His
              165             170             175


Pro Ala Trp Ser Ala Pro Arg Arg Arg Ala Met Asp Leu Cys Thr Phe
              180             185             190


Val Phe Ser Tyr Leu Leu Pro Val Leu Val Leu Ser Leu Thr Tyr Ala
              195             200             205


Arg Thr Leu Arg Tyr Leu Trp Arg Thr Val Asp Pro Val Thr Ala Gly
    210             215             220


Ser Gly Ser Gln Arg Ala Lys Arg Lys Val Thr Arg Met Ile Ile Ile
225             230             235             240


Val Ala Val Leu Phe Cys Leu Cys Trp Met Pro His His Ala Leu Ile
              245             250             255


Leu Cys Val Trp Phe Gly Arg Phe Pro Leu Thr Arg Ala Thr Tyr Ala
              260             265             270


Leu Arg Ile Leu Ser His Leu Val Ser Tyr Ala Asn Ser Cys Val Asn
          275             280             285


Pro Ile Val Tyr Ala Leu Val Ser Lys His Phe Arg Lys Gly Phe Arg
    290             295             300
```

Lys Ile Cys Ala Gly Leu Leu Arg Pro Ala Pro Arg Arg Ala Ser Gly
305   310   315   320

Arg Val Ser Ile Leu Ala Pro Gly Asn His Ser Gly Ser Met Leu Glu
            325   330   335

Gln Glu Ser Thr Asp Leu Thr Gln Val Ser Glu Ala Ala Gly Pro Leu
            340   345   350

Val Pro Pro Pro Ala Leu Pro Asn Cys Thr Ala Ser Ser Arg Thr Leu
            355   360   365

Asp Pro Ala Cys   *
    370

(2) INFORMATION FOR SEQ ID NO:9:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 2200 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: DNA

        (ix) FEATURE:
            (A) NAME/KEY: CDS
            (B) LOCATION: 46..414

        (ix) FEATURE:
            (A) NAME/KEY: CDS
            (B) LOCATION: 1422..2171

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CAAGACCCGG ACAGCTGCGG GAGCGGCGTC CACTTTGGTG ATACC ATG AAT GGC   54
                                              Met Asn Gly
                                              1

- 143 -

```
TCC GGC AGC CAG GGC GCG GAG AAC ACG AGC CAG GAA GGC GGT AGC GGC 102
Ser Gly Ser Gln Gly Ala Glu Asn Thr Ser Gln Glu Gly Gly Ser Gly
        5                   10                  15


GGC TGG CAG CCT GAG GCG GTC CTT GTA CCC CTA TTT TTC GCG CTC ATC 150
Gly Trp Gln Pro Glu Ala Val Leu Val Pro Leu Phe Phe Ala Leu Ile
 20                  25                  30                  35


TTC CTC GTG GGC ACC GTG GGC AAC GCG CTG GTG CTG GCG GTG CTG CTG 198
Phe Leu Val Gly Thr Val Gly Asn Ala Leu Val Leu Ala Val Leu Leu
            40                  45                  50


CGC GGC GGC CAG GCG GTC AGC ACC ACC AAC CTG TTC ATC CTC AAC CTG 246
Arg Gly Gly Gln Ala Val Ser Thr Thr Asn Leu Phe Ile Leu Asn Leu
            55                  60                  65


GGC GTG GCC GAC CTG TGT TTC ATC CTG TGC TGC GTG CCT TTC CAG GCC 294
Gly Val Ala Asp Leu Cys Phe Ile Leu Cys Cys Val Pro Phe Gln Ala
            70                  75                  80


ACC ATC TAC ACC CTG GAC GAC TGG GTG TTC GGC TCG CTG CTC TGC AAG 342
Thr Ile Tyr Thr Leu Asp Asp Trp Val Phe Gly Ser Leu Leu Cys Lys
        85                  90                  95


GCT GTT CAT TTC CTC ATC TTT CTC ACT ATG CAC GCC AGC AGC TTC ACG 390
Ala Val His Phe Leu Ile Phe Leu Thr Met His Ala Ser Ser Phe Thr
100                 105                 110                 115


CTG GCC GCC GTC TCC CTG GAC AGG TGAGTGAACA TCGGAGAACT ATTGTATCTG 444
Leu Ala Ala Val Ser Leu Asp Arg
                120


AGATAGGGGCTTGGGCTGGAGTCACTACACAGGGGATCCAGAAGGCATGAGCAGAATGGG      504

CGAGAACACTGAAATTACAAAGTGGCCTGAGGCCGTGAAACGCAAGGGGGAGGGAGATTA      564

AGACTCAGTGACTGAGAGTGTCTAAGTCGATGGGAGAAATCGGGTCTCTGGGGTCCTCGC      624

ATTATTACTGCTTGAGTTAAATGTCTCTGTGAAACATTGCAGTTCTCAGGCCAGAGTTGG      684

CAGGAAAAGTAACTCGCCAGTGTTCAGATGCTGTTTGAGAGCTGCAGAGAAGCATCTGCT      744
```

- 144 -

TCTTAGCACCAAGCTCAGCACCTGGGGCGTTGTCCGGCGCCTTAGGCTTAGGACTGGGCT   804

GTGCTGTGTTAAGACCCATGCTCAAGTCCAACGGAGTGTAAGCGAGGGCTCCTAGCTGAC   864

ACCCAGAGCCCTCCAGGCCAAGGCTCCCCTCACCGAGATGCCAGCGGTTTTATGCTCCTT   924

CCATAGGTAAAGGACCCAGAAAGAAACATCCAGTATGCCCGGAGGGATCTTGACTGGAAA   984

AGACTGAATCCTGGTCTGGTGACCTTAGTTCCCTGCCCTTTCACATCACTTGGACATTCC   1044

CACAGAAGAGCGGTGAAGAGGCGGTGGTCCTTATTCTCCTCTGGTTTCCACTGAGTGCAA   1104

CATGTGCGTCCTGAGTACGCTGGAGGGACTCACAAAATTTCAGCTTTCTT TAGGAGTTTC   1164

CTTGCTGTAGTTTGACCCAAGTCTTCTCCAGGTTTCTGTCAGAACCTCAG GCATGAGGGA   1224

TCTGCCTCCCCTGGTTGTCACCAGAGGATAACAATCACTGCCCCCAGAAATCCAGACAGA   1284

TTCTACAACTTTTAGTCTTCGGTGTTTTGGGGGTGCCCCTTCACGTGGAG TAGGTCGGTG   1344

GCCACATTCCCAGGAGTGACAATAGCCTAGCAGTGAATCCTCTCGCTTAG CTGATGCCCC   1404

CCCACTGTCCCCACAGG TAT CTG GCC ATC CGC TAC CCG CTG CAC TCC CGA   1454
                   Tyr Leu Ala Ile Arg Tyr Pro Leu His Ser Arg
                    1   5   10

GAG TTG CGC ACA CCT CGA AAC GCG CTG GCC GCC ATC GGG .CTC ATC TGG   1502
Glu Leu Arg Thr Pro Arg Asn Ala Leu Ala Ala Ile Gly Leu Ile Trp
         15   20   25

GGG CTA GCA CTG CTC TTC TCC GGG CCC TAC CTG AGC TAC TAC CGT CAG   1550
Gly Leu Ala Leu Leu Phe Ser Gly Pro Tyr Leu Ser Tyr Tyr Arg Gln
     ·   30   35   40

TCG CAG CTG GCC AAC CTG ACA GTA TGC CAC CCA GCA TGG AGC GCA CCT   1598
Ser Gln Leu Ala Asn Leu Thr Val Cys His Pro Ala Trp Ser Ala Pro
     45   50   55

CGA CGT CGA GCC ATG GAC CTC TGC ACC TTC GTC TTT AGC TAC CTG CTG   1646
Arg Arg Arg Ala Met Asp Leu Cys Thr Phe Val Phe Ser Tyr Leu Leu
 60   65   70   75

```
CCA GTG CTA GTC CTC AGT CTG ACC TAT GCG CGT ACC CTG CGC TAC CTC 1694
Pro Val Leu Val Leu Ser Leu Thr Tyr Ala Arg Thr Leu Arg Tyr Leu
            80              85                  90


TGG CGC ACA GTC GAC CCG GTG ACT GCA GGC TCA GGT TCC CAG CGC GCC 1742
Trp Arg Thr Val Asp Pro Val Thr Ala Gly Ser Gly Ser Gln Arg Ala
            95              100                 105


AAA CGC AAG GTG ACA CGG ATG ATC ATC ATC GTG GCG GTG CTT TTC TGC 1790
Lys Arg Lys Val Thr Arg Met Ile Ile Ile Val Ala Val Leu Phe Cys
            110             115                 120


CTC TGT TGG ATG CCC CAC CAC GCG CTT ATC CTC TGC GTG TGG TTT GGT 1838
Leu Cys Trp Met Pro His His Ala Leu Ile Leu Cys Val Trp Phe Gly
        125             130                 135


CGC TTC CCG CTC ACG CGT GCC ACT TAC GCG TTG CGC ATC CTT TCA CAC 1886
Arg Phe Pro Leu Thr Arg Ala Thr Tyr Ala Leu Arg Ile Leu Ser His
140             145                 150                 155


CTA GTT TCC TAT GCC AAC TCC TGT GTC AAC CCC ATC GTT TAC GCT CTG 1934
Leu Val Ser Tyr Ala Asn Ser Cys Val Asn Pro Ile Val Tyr Ala Leu
            160             165                 170


GTC TCC AAG CAT TTC CGT AAA GGT TTC CGC AAA ATC TGC GCG GGC CTG 1982
Val Ser Lys His Phe Arg Lys Gly Phe Arg Lys Ile Cys Ala Gly Leu
            175             180                 185


CTG CGC CCT GCC CCG AGG CGA GCT TCG GGC CGA GTG AGC ATC CTG GCG 2030
Leu Arg Pro Ala Pro Arg Arg Ala Ser Gly Arg Val Ser Ile Leu Ala
            190             195                 200


CCT GGG AAC CAT AGT GGC AGC ATG CTG GAA CAG GAA TCC ACA GAC CTG 2078
Pro Gly Asn His Ser Gly Ser Met Leu Glu Gln Glu Ser Thr Asp Leu
            205             210                 215


ACA CAG GTG AGC GAG GCA GCC GGG CCC CTT GTC CCA CCA CCC GCA CTT 2126
Thr Gln Val Ser Glu Ala Ala Gly Pro Leu Val Pro Pro Pro Ala Leu
220             225                 230                 235
```

CCC AAC TGC ACA GCC TCG AGT AGA ACC CTG GAT CCG GCT TGT TAA   2171
Pro Asn Cys Thr Ala Ser Ser Arg Thr Leu Asp Pro Ala Cys *
            240   245   250

AGGACCAAAG GGCATCTAAC AGCTTCTAG   2200

(2) INFORMATION FOR SEQ ID NO:10:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 123 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Asn Gly Ser Gly Ser Gln Gly Ala Glu Asn Thr Ser Gln Glu Gly
 1   5   10   15

Gly Ser Gly Gly Trp Gln Pro Glu Ala Val Leu Val Pro Leu Phe Phe
            20   25   30

Ala Leu Ile Phe Leu Val Gly Thr Val Gly Asn Ala Leu Val Leu Ala
            35   40   45

Val Leu Leu Arg Gly Gly Gln Ala Val Ser Thr Thr Asn Leu Phe Ile
    50   55   60

Leu Asn Leu Gly Val Ala Asp Leu Cys Phe Ile Leu Cys Cys Val Pro
65   70   75   80

Phe Gln Ala Thr Ile Tyr Thr Leu Asp Asp Trp Val Phe Gly Ser Leu
                85   90   95

Leu Cys Lys Ala Val His Phe Leu Ile Phe Leu Thr Met His Ala Ser
            100   105   110

Ser Phe Thr Leu Ala Ala Val Ser Leu Asp Arg
            115   120

(2) INFORMATION FOR SEQ ID NO:11:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 250 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Tyr Leu Ala Ile Arg Tyr Pro Leu His Ser Arg Glu Leu Arg Thr Pro
 1               5                  10                  15

Arg Asn Ala Leu Ala Ala Ile Gly Leu Ile Trp Gly Leu Ala Leu Leu
            20                  25                  30

Phe Ser Gly Pro Tyr Leu Ser Tyr Tyr Arg Gln Ser Gln Leu Ala Asn
            35                  40                  45

Leu Thr Val Cys His Pro Ala Trp Ser Ala Pro Arg Arg Arg Ala Met
        50                  55                  60

Asp Leu Cys Thr Phe Val Phe Ser Tyr Leu Leu Pro Val Leu Val Leu
65                  70                  75                  80

Ser Leu Thr Tyr Ala Arg Thr Leu Arg Tyr Leu Trp Arg Thr Val Asp
                85                  90                  95

Pro Val Thr Ala Gly Ser Gly Ser Gln Arg Ala Lys Arg Lys Val Thr
                100                 105                 110

Arg Met Ile Ile Ile Val Ala Val Leu Phe Cys Leu Cys Trp Met Pro
        115                 120                 125

His His Ala Leu Ile Leu Cys Val Trp Phe Gly Arg Phe Pro Leu Thr
    130                 135                 140

Arg Ala Thr Tyr Ala Leu Arg Ile Leu Ser His Leu Val Ser Tyr Ala
145                 150                 155                 160

Asn Ser Cys Val Asn Pro Ile Val Tyr Ala Leu Val Ser Lys His Phe
```

- 148 -

```
                      165                  170                  175


      Arg Lys Gly Phe Arg Lys Ile Cys Ala Gly Leu Leu Arg Pro Ala Pro
              180                  185                  190


      Arg Arg Ala Ser Gly Arg Val Ser Ile Leu Ala Pro Gly Asn His Ser
              195                  200                  205


      Gly Ser Met Leu Glu Gln Glu Ser Thr Asp Leu Thr Gln Val Ser Glu
          210                  215                  220


      Ala Ala Gly Pro Leu Val Pro Pro Pro Ala Leu Pro Asn Cys Thr Ala
      225                  230                  235                  240


      Ser Ser Arg Thr Leu Asp Pro Ala Cys *
                  245                  250
```

(2) INFORMATION FOR SEQ ID NO:12:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 26 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CAAGGCTGTT CATTTCCTCA TCTTTC   26

(2) INFORMATION FOR SEQ ID NO:13:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 25 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TTGGAGACCA GAGCGTAAAC GATGG   .   25

(2) INFORMATION FOR SEQ ID NO:14:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 45 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: DNA

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AGTCGACCCG GTGACTGCAG GCTCAGGTTC CCAGCGCGCC AAACG   45 .

(2) INFORMATION FOR SEQ ID NO:15:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 23 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: DNA

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CCCTACCTGA GCTACTACCG TCA   23

- 150 -

(2) INFORMATION FOR SEQ ID NO:16:

    (i) SEQUENCE CHARACTERISTICS:

       (A) LENGTH: 24 base pairs

       (B) TYPE: nucleic acid

       (C) STRANDEDNESS: single

       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ACCAAACCAC ACGCAGAGGA TAAG   24

(2) INFORMATION FOR SEQ ID NO:17:

    (i) SEQUENCE CHARACTERISTICS:

       (A) LENGTH: 26 base pairs

       (B) TYPE: nucleic acid

       (C) STRANDEDNESS: single

       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CCACGATGAG GATCATGCGT GTCACC   26

(2) INFORMATION FOR SEQ ID NO:18:

    (i) SEQUENCE CHARACTERISTICS:

       (A) LENGTH: 26 base pairs

       (B) TYPE: nucleic acid

       (C) STRANDEDNESS: single

       (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TAGGTCAGGC CGAGAACCAG CACAGG   26

(2) INFORMATION FOR SEQ ID NO:19:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 24 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CAGGTAGCTG AAGACGAAGG TGCA   24

(2) INFORMATION FOR SEQ ID NO:20:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 25 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CTGCACCTTC GTCTTCAGCT ACCTG   25

(2) INFORMATION FOR SEQ ID NO:21:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 26 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CCTGTGCTGG TTCTCGGCCT GACCTA   26

(2) INFORMATION FOR SEQ ID NO:22:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 26 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TATCTGGCCA TCCGCTACCC GCTGCA   26

(2) INFORMATION FOR SEQ ID NO:23:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 45 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

- 153 -

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TTGCGCTACC TCTGGCGCGC CGTCGACCCG GTGGCCGCGG GCTCG   45

(2) INFORMATION FOR SEQ ID NO:24:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 25 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CCAACAATGA CTCCAACTCT GTGAC   25

(2) INFORMATION FOR SEQ ID NO:25:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 25 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

AGGCGCAGAA CTGGTAGGTA TGGAA   25

(2) INFORMATION FOR SEQ ID NO:26:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 25 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

AAGCTTCTAG AGATCCCTCG ACCTC   25

(2) INFORMATION FOR SEQ ID NO:27:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 28 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ACGGAATTCG ACATGAATGG CTCCGGCA   28

(2) INFORMATION FOR SEQ ID NO:28:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 34 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GCTCTAGAGC CCCTTTGGTC CTTTAACAAG CCGG   34

(2) INFORMATION FOR SEQ ID NO:29:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 1365 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 102..1265

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

AGTCGCACTA GGAGTTGCAG CGGCCGCAGC CCCGGGAGCT TCCCGCTCGC GGAGACCCAG 60

ACGGCTGCAG GAGCCCGGGC AGCCTCGGGG TCAGCGGCAC C ATG AAC GTC TCG   113
                                              Met Asn Val Ser
                                               1

GGC TGC CCA GGG GCC GGG AAC GCG AGC CAG GCG GGC GGC GGG GGA GGC 161
Gly Cys Pro Gly Ala Gly Asn Ala Ser Gln Ala Gly Gly Gly Gly Gly
 5   10   15   20

TGG CAC CCC GAG GCG GTC ATC GTG CCC CTG CTC TTC GCG CTC ATC TTC 209
Trp His Pro Glu Ala Val Ile Val Pro Leu Leu Phe Ala Leu Ile Phe
            25   30   35

- 156 -

```
CTC GTG GGC ACC GTG GGC AAC ACG CTG GTG CTG GCG GTG CTG CTG CGC 257
Leu Val Gly Thr Val Gly Asn Thr Leu Val Leu Ala Val Leu Leu Arg
        40              45              50


GGC GGC CAG GCG GTC AGC ACT ACC AAC CTG TTC ATC CTT AAC CTG GGC 305
Gly Gly Gln Ala Val Ser Thr Thr Asn Leu Phe Ile Leu Asn Leu Gly
        55              60              65


GTG GCC GAC CTG TGT TTC ATC CTG TGC TGC GTG CCC TTC CAG GCC ACC 353
Val Ala Asp Leu Cys Phe Ile Leu Cys Cys Val Pro Phe Gln Ala Thr
        70              75              80


ATC TAC ACC CTG GAC GGC TGG GTG TTC GGC TCG CTG CTG TGC AAG GCG 401
Ile Tyr Thr Leu Asp Gly Trp Val Phe Gly Ser Leu Leu Cys Lys Ala
 85              90              95              100


GTG CAC TTC CTC ATC TTC CTC ACC ATG CAC GCC AGC AGC TTC ACG CTG 449
Val His Phe Leu Ile Phe Leu Thr Met His Ala Ser Ser Phe Thr Leu
            105             110             115


GCC GCC GTC TCC CTG GAC AGG TAT CTG GCC ATC CGC TAC CCG CTG CAC 497
Ala Ala Val Ser Leu Asp Arg Tyr Leu Ala Ile Arg Tyr Pro Leu His
            120             125             130


TCC CGC GAG CTG CGC ACG CCT CGA AAC GCG CTG GCA GCC ATC GGG CTC 545
Ser Arg Glu Leu Arg Thr Pro Arg Asn Ala Leu Ala Ala Ile Gly Leu
        135             140             145


ATC TGG GGG CTG TCG CTG CTC TTC TCC GGG CCC TAC CTG AGC TAC TAC 593
Ile Trp Gly Leu Ser Leu Leu Phe Ser Gly Pro Tyr Leu Ser Tyr Tyr
    150             155             160


CGC CAG TCG CAG CTG GCC AAC CTG ACC GTG TGC CAT CCC GCG TGG AGC 641
Arg Gln Ser Gln Leu Ala Asn Leu Thr Val Cys His Pro Ala Trp Ser
165             170             175             180


GCC CCT CGC CGC CGC GCC ATG GAC ATC TGC ACC TTC GTC TTC AGC TAC 689
Ala Pro Arg Arg Arg Ala Met Asp Ile Cys Thr Phe Val Phe Ser Tyr
            185             190             195
```

```
CTG CTT CCT GTG CTG GTT CTC GGC CTG ACC TAC GCG CGC ACC TTG CGC 737
Leu Leu Pro Val Leu Val Leu Gly Leu Thr Tyr Ala Arg Thr Leu Arg
         200                 205                 210


TAC CTC TGG CGC GCC GTC GAC CCG GTG GCC GCG GGC TCG GGT GCC CGG 785
Tyr Leu Trp Arg Ala Val Asp Pro Val Ala Ala Gly Ser Gly Ala Arg
         215                 220                 225


CGC GCC AAG CGC AAG GTG ACA CGC ATG ATC CTC ATC GTG GCC GCG CTC 833
Arg Ala Lys Arg Lys Val Thr Arg Met Ile Leu Ile Val Ala Ala Leu
         230                 235                 240


TTC TGC CTC TGC TGG ATG CCC CAC CAC GCG CTC ATC CTC TGC GTG TGG 881
Phe Cys Leu Cys Trp Met Pro His His Ala Leu Ile Leu Cys Val Trp
245                 250                 255                 260


TTC GGC CAG TTC CCG CTC ACG CGC GCC ACT TAT GCG CTT CGC ATC CTC 929
Phe Gly Gln Phe Pro Leu Thr Arg Ala Thr Tyr Ala Leu Arg Ile Leu
         265                 270                 275


TCG CAC CTG GTC TCC TAC GCC AAC TCC TGC GTC AAC CCC ATC GTT TAC 977
Ser His Leu Val Ser Tyr Ala Asn Ser Cys Val Asn Pro Ile Val Tyr
         280                 285                 290


GCG CTG GTC TCC AAG CAC TTC CGC AAA GGC TTC CGC ACG ATC TGC GCG 1025
Ala Leu Val Ser Lys His Phe Arg Lys Gly Phe Arg Thr Ile Cys Ala
         295                 300                 305


GGC CTG CTG GGC CGT GCC CCA GGC CGA GCC TCG GGC CGT GTG TGC GCT 1073
Gly Leu Leu Gly Arg Ala Pro Gly Arg Ala Ser Gly Arg Val Cys Ala
         310                 315                 320


GCC GCG CGG GGC ACC CAC AGT GGC AGC GTG TTG GAG CGC GAG TCC AGC 1121
Ala Ala Arg Gly Thr His Ser Gly Ser Val Leu Glu Arg Glu Ser Ser
325                 330                 335                 340


GAC CTG TTG CAC ATG AGC GAG GCG GCG GGG GCC CTT CGT CCC TGC CCC 1169
Asp Leu Leu His Met Ser Glu Ala Ala Gly Ala Leu Arg Pro Cys Pro
         345                 350                 355
```

```
GGC GCT TCC CAG CCA TGC ATC CTC GAG CCC TGT CCT GGC CCG TCC TGG 1217
Gly Ala Ser Gln Pro Cys Ile Leu Glu Pro Cys Pro Gly Pro Ser Trp
          360             365             370
```

```
CAG GGC CCA AAG GCA GGC GAC AGC ATC CTG ACG GTT GAT GTG GCC TGA 1265
Gln Gly Pro Lys Ala Gly Asp Ser Ile Leu Thr Val Asp Val Ala  *
          375             380             385
```

```
AAGCACTTAG CGGGCGCGCTGGGATGTCACAGAGTTGGAGTCATTGTTGGGGGACCGTGG      1325
```

```
GGAGAGCTTTGCCTGTTAATAAAACGCACAAACCATTTCA                           1365
```

(2) INFORMATION FOR SEQ ID NO:30:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 388 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
Met Asn Val Ser Gly Cys Pro Gly Ala Gly Asn Ala Ser Gln Ala Gly
  1             5              10              15
```

```
Gly Gly Gly Gly Trp His Pro Glu Ala Val Ile Val Pro Leu Leu Phe
          20              25              30
```

```
Ala Leu Ile Phe Leu Val Gly Thr Val Gly Asn Thr Leu Val Leu Ala
          35              40              45
```

```
Val Leu Leu Arg Gly Gly Gln Ala Val Ser Thr Thr Asn Leu Phe Ile
  50              55              60
```

```
Leu Asn Leu Gly Val Ala Asp Leu Cys Phe Ile Leu Cys Cys Val Pro
 65              70              75              80
```

```
Phe Gln Ala Thr Ile Tyr Thr Leu Asp Gly Trp Val Phe Gly Ser Leu
              85              90              95
```

```
Leu Cys Lys Ala Val His Phe Leu Ile Phe Leu Thr Met His Ala Ser
        100                 105                 110

Ser Phe Thr Leu Ala Ala Val Ser Leu Asp Arg Tyr Leu Ala Ile Arg
        115                 120                 125

Tyr Pro Leu His Ser Arg Glu Leu Arg Thr Pro Arg Asn Ala Leu Ala
        130                 135                 140

Ala Ile Gly Leu Ile Trp Gly Leu Ser Leu Leu Phe Ser Gly Pro Tyr
145                 150                 155                 160

Leu Ser Tyr Tyr Arg Gln Ser Gln Leu Ala Asn Leu Thr Val Cys His
                165                 170                 175

Pro Ala Trp Ser Ala Pro Arg Arg Arg Ala Met Asp Ile Cys Thr Phe
            180                 185                 190

Val Phe Ser Tyr Leu Leu Pro Val Leu Val Leu Gly Leu Thr Tyr Ala
        195                 200                 205

Arg Thr Leu Arg Tyr Leu Trp Arg Ala Val Asp Pro Val Ala Ala Gly
     210                 215                 220

Ser Gly Ala Arg Arg Ala Lys Arg Lys Val Thr Arg Met Ile Leu Ile
225                 230                 235                 240

Val Ala Ala Leu Phe Cys Leu Cys Trp Met Pro His His Ala Leu Ile
                245                 250                 255

Leu Cys Val Trp Phe Gly Gln Phe Pro Leu Thr Arg Ala Thr Tyr Ala
            260                 265                 270

Leu Arg Ile Leu Ser His Leu Val Ser Tyr Ala Asn Ser Cys Val Asn
        275                 280                 285

Pro Ile Val Tyr Ala Leu Val Ser Lys His Phe Arg Lys Gly Phe Arg
    290                 295                 300

Thr Ile Cys Ala Gly Leu Leu Gly Arg Ala Pro Gly Arg Ala Ser Gly
305                 310                 315                 320
```

```
Arg Val Cys Ala Ala Ala Arg Gly Thr His Ser Gly Ser Val Leu Glu
            325                 330                 335


Arg Glu Ser Ser Asp Leu Leu His Met Ser Glu Ala Ala Gly Ala Leu
            340                 345                 350


Arg Pro Cys Pro Gly Ala Ser Gln Pro Cys Ile Leu Glu Pro Cys Pro
            355                 360                 365


Gly Pro Ser Trp Gln Gly Pro Lys Ala Gly Asp Ser Ile Leu Thr Val
    370                 375                 380


Asp Val Ala   *
385
```

(2) INFORMATION FOR SEQ ID NO:31:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 1384 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
GTGAGCCAGCGCCTTGGCCTCCCTGGGAGATGGGCATCCACGCGGGGGATGGAGCGGGAG        60

GCGGGACTGGGGACCAAGAAGGGACGCGCAGAGTGGGACAGGACACTAAG AAGGCAGTGG       120

AAGACAAGCGGGCGCGGAGGAGGAAAAAGAGGAATAAGAATGGGGGACCG TGGTGTCCCT       180

CGGTTAGATGCGTCCTGGGGCCTGGAAGCCTGGAGAATGTGGCTCTCCAGCGCCGCCCGT       240

GCCTGACAACGCGCAGCGTTTCCCAGTACGACGCGTTTGTGCGCGTTCATCTCGCTTGAG       300

CTTAATGCCCTCCGTGAGGGTGGGATAGGACAAAGTGCCCAATATACAGA AGAGTTGAGT       360

TCCTAAGTAACTCGCTCAGAGTCGCCAGCCAGGGATCGGGTGCGTGAAGTGACCGTCTGT       420
```

CTCCTGCAGCCAACTTCAGGCGCCTCCACTGCGCTCGCCTCCAAGCCACGGTTTGGTTGG   480

TTGGTGCAGCTGGCTCAGGTCCAGGCTGTGGATCTTGGGTCCTTTGCAAGGATCCACTCC   540

GGAGTCCCAGCGAGCGTGCCTAAAGGTCCCTAGCTCAGTCCCAGCCCACTCTGCCTCTCG   600

CCTCCAAACAAAACAAAACAAAATAAAATCCAAAACAAGTCGGGGCCGGGAGAGGAGCGT   660

GCCCTGGGGTTCTTCCTCCCCAGCCAGAGGAGAGCGAGAGACGCACATTCGGGAGAGCGC   720

GGGACTCAGGTGGAGCTTGAAAGGACACTGGGATGGTTCCTGGGGAGGAAATCCGGGTAT   780

TTCCCCTCTCCATCCTCTGGAAAAACAGAGAGGCGAGGCCAGACTGCCCCCACACCTCCT   840

GTAGCCACTGAGCGCGAAGTGCGTTGGTTCCGAGCGCGCTGGTGGGATCCACAAAGCTCG   900

CATTCTCTCAGGAATCCCCTGAGAAATTAACTGTCCCTTGCCCAACATGTCTTCTCCAGG   960

CTGTCTGCTAGAGCCTCAGGCGCCTCCGCCCTCCCTCCCGCGGCACCGTCACCAGTGGGT   1020

AGTCACAGCCTCCCGGAGCCCATAGCCGGTTCTCCAACCTTTAGTCTTCAGTGGCTTTGG   1080

GGTGCCCTCTCAGTGGAGACTGTGGTTGCAGTCCCCGGGGGCAGCGGGAGAATGGCTTGA   1140

AGGCACACCTTTCCTGCTGCCGGGCCCGCCCCATTTCCAGGCTCCGCTGAGTGTCTGGGA   1200

CACGCTGGGAGGCCCCCACCTCCGCCCTCACGCCGAGCCTCACCCCCACCTCCTCTGTGT   1260

GCGGTGTAACCATGCGCTAAGGACCTTCCTCGAGAGCAGCCTTGGGACCGAGGTGCAGGG   1320

GTCGCGGCCCTCCAGCATGAATGTGCCCGCTCAGCCGACGTCTCCCTTCCCGGTCTGACC   1380

GCAG   1384

(2) INFORMATION FOR SEQ ID NO:32:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 346 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Met Glu Leu Ala Pro Val Asn Leu Ser Glu Gly Asn Gly Ser Asp Pro
1   5   10   15

Glu Pro Pro Ala Glu Pro Arg Pro Leu Phe Gly Ile Gly Val Glu Asn
                20   25   30

Phe Ile Thr Leu Val Val Phe Gly Leu Ile Phe Ala Met Gly Val Leu
            35   40   45

Gly Asn Ser Leu Val Ile Thr Val Leu Ala Arg Ser Lys Pro Gly Lys
        50   55   60

Pro Arg Ser Thr Thr Asn Leu Phe Ile Leu Asn Leu Ser Ile Ala Asp
65   70   75   80

Leu Ala Tyr Leu Leu Phe Cys Ile Pro Phe Gln Ala Thr Val Tyr Ala
                85   90   95

Leu Pro Thr Trp Val Leu Gly Ala Phe Ile Cys Lys Phe Ile His Tyr
                100   105   110

Phe Phe Thr Val Ser Met Leu Val Ser Ile Phe Thr Leu Ala Ala Met
            115   120   125

Ser Val Asp Arg Tyr Val Ala Ile Val His Ser Arg Arg Ser Ser Ser
        130   135   140

- 163 -

```
Leu Arg Val Ser Arg Asn Ala Leu Leu Gly Val Gly Phe Ile Trp Ala
145             150             155             160


Leu Ser Ile Ala Met Ala Ser Pro Val Ala Tyr Tyr Gln Arg Leu Phe
            165             170             175



His Arg Asp Ser Asn Gln Thr Phe Cys Trp Glu His Trp Pro Asn Gln
            180             185             190



Leu His Lys Lys Ala Tyr Val Val Cys Thr Phe Val Phe Gly Tyr Leu
            195             200             205



Leu Pro Leu Leu Leu Ile Cys Phe Cys Tyr Ala Lys Val Leu Asn His
    210             215             220



Leu His Lys Lys Leu Lys Asn Met Ser Lys Lys Ser Glu Ala Ser Lys
225             230             235             240


Lys Lys Thr Ala Gln Thr Val Leu Val Val Val Val Phe Gly Ile
            245             250             255



Ser Trp Leu Pro His His Val Ile His Leu Trp Ala Glu Phe Gly Ala
            260             265             270



Phe Pro Leu Thr Pro Ala Ser Phe Phe Phe Arg Ile Thr Ala His Cys
            275             280             285



Leu Ala Tyr Ser Asn Ser Ser Val Asn Pro Ile Ile Tyr Ala Phe Leu
    290             295             300



Ser Glu Asn Phe Arg Lys Ala Tyr Lys Gln Val Phe Lys Cys Arg Val
305             310             315             320
```

Cys Asn Glu Ser Pro His Gly Asp Ala Lys Glu Lys Asn Arg Ile Asp
                325   330   335

Thr Pro Pro Ser Thr Asn Cys Thr His Val
        340   345

What is claimed is:

1.   A nucleic acid encoding a GALR2 receptor.

2.   The nucleic acid of claim 1, wherein the nucleic acid is DNA.

3.   The DNA of claim 2, wherein the DNA is cDNA.

4.   The DNA of claim 2, wherein the DNA is genomic DNA.

5.   The nucleic acid of claim 1, wherein the nucleic acid is RNA.

6.   The nucleic acid of claim 1, wherein the nucleic acid encodes a vertebrate GALR2 receptor.

7.   The nucleic acid of claim 1, wherein the nucleic acid encodes a mammalian GALR2 receptor.

8.   The nucleic acid of claim 1, wherein the nucleic acid encodes a rat GALR2 receptor.

9.   The nucleic acid of claim 1, wherein the nucleic acid encodes a human GALR2 receptor.

10.   The nucleic acid of claim 7, wherein the nucleic acid encodes a receptor characterized by an amino acid sequence in the transmembrane region which has a homology of 60% or higher to the amino acid sequence in the transmembrane region of the rat GALR2 receptor and a homology of less than 60% to the amino acid sequence in the transmembrane region of any GALR1 receptor.

11. The nucleic acid of claim 7, wherein the nucleic
     acid encodes a mammalian GALR2 receptor which has
     substantially the same amino acid sequence as does
     the GALR2 receptor encoded by the plasmid K985 (ATCC
5   Accession No. 97426).

12. The nucleic acid of claim 8, wherein the nucleic
     acid encodes a rat GALR2 receptor which has an amino
     acid sequence encoded by the plasmid K985 (ATCC
10   Accession No. 97426).

13. The nucleic acid of claim 7, wherein the nucleic
     acid encodes a mammalian GALR2 receptor which has
     substantially the same amino acid sequence as does
15   the GALR2 receptor encoded by the plasmid K1045
     (ATCC Accession No. 97778).

14. The nucleic acid of claim 8, wherein the nucleic
     acid encodes a rat GALR2 receptor which has an amino
20   acid sequence encoded by the plasmid K1045 (ATCC
     Accession No. 97778).

15. The nucleic acid of claim 8, wherein the nucleic
     acid encodes a rat GALR2 receptor which has
25   substantially the same amino acid sequence as that
     shown in Figure 2.

16. The nucleic acid of claim 8, wherein the nucleic
     acid encodes a rat GALR2 receptor which has the
30   amino acid sequence shown in Figure 2.

17. The nucleic acid of claim 7, wherein the nucleic
     acid encodes a mammalian GALR2 receptor which has
     substantially the same amino acid sequence as does
35   the GALR2 receptor encoded by the plasmid BO29 (ATCC

Accession No. 97735).

18.   The nucleic acid of claim 9, wherein the nucleic
      acid encodes a human GALR2 receptor which has an
      amino acid sequence encoded by the plasmid BO29
      (ATCC Accession No. 97735).

19.   The nucleic acid of claim 7, wherein the nucleic
      acid encodes a mammalian GALR2 receptor which has
      substantially the same amino acid sequence as does
      the GALR2 receptor encoded by the plasmid BO39 (ATCC
      Accession No. _____).

20.   The nucleic acid of claim 9, wherein the nucleic
      acid encodes a human GALR2 receptor which has an
      amino acid sequence encoded by the plasmid BO39
      (ATCC Accession No. _____).

21.   The nucleic acid of claim 9, wherein the nucleic
      acid encodes a human GALR2 receptor which has
      substantially the same amino acid sequence as that
      shown in Figure 11.

22.   The nucleic acid of claim 9, wherein the nucleic
      acid encodes a human GALR2 receptor which has the
      amino acid sequence shown in Figure 11.

23.   An isolated nucleic acid encoding a modified GALR2
      receptor, which differs from a GALR2 receptor by
      having an amino acid(s) deletion, replacement or
      addition in the third intracellular domain.

24.   The nucleic acid of claim 23, wherein the modified
      GALR2 receptor differs from a GALR2 receptor by
      having a deletion in the third intracellular domain.

25.   The nucleic acid of claim 23, wherein the modified GALR2 receptor differs from a GALR2 receptor by having a replacement or addition in the third intracellular domain.

26.   A purified GALR2 receptor protein.

27.   A vector comprising the nucleic acid of claim 1.

28.   A vector comprising the nucleic acid of either of claims 8 or 9.

29.   A vector of claim 27 adapted for expression in a bacterial cell which comprises the regulatory elements necessary for expression of the nucleic acid in the bacterial cell operatively linked to the nucleic acid encoding a GALR2 receptor as to permit expression thereof.

30.   A vector of claim 27 adapted for expression in a yeast cell which comprises the regulatory elements necessary for expression of the nucleic acid in the yeast cell operatively linked to the nucleic acid encoding a GALR2 receptor as to permit expression thereof.

31.   A vector of claim 27 adapted for expression in an insect cell which comprises the regulatory elements necessary for expression of the nucleic acid in the insect cell operatively linked to the nucleic acid encoding the GALR2 receptor as to permit expression thereof.

32.   A vector of claim 31 which is a baculovirus.

33. A vector of claim 27 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding a GALR2 receptor as to permit expression thereof.

34. A vector of claim 27 wherein the vector is a plasmid.

35. The plasmid of claim 34 designated K985 (ATCC Accession No. 97426).

36. The plasmid of claim 34 designated K1045 (ATCC Accession No. 97778).

37. The plasmid of claim 34 designated BO29 (ATCC Accession No. 97735).

38. The plasmid of claim 34 designated BO39 (ATCC Accession No. _____).

39. A cell comprising the vector of claim 27.

40. A cell of claim 39, wherein the cell is a non-mammalian cell.

41. A cell of claim 40, wherein the non-mammalian cell is a Xenopus oocyte cell or a Xenopus melanophore cell.

42. A cell of claim 39, wherein the cell is a mammalian cell.

43. A mammalian cell of claim 42, wherein the cell is a

- 170 -

COS-7 cell, a 293 human embryonic kidney cell, a
NIH-3T3 cell, a LM(tk-) cell or a CHO cell.

44. The LM(tk-) cell of claim 43 designated L-rGALR2-8
    (ATCC Accession No. CRL-12074).

45. The LM(tk-) cell of claim 43 designated L-rGALR2I-4
    (ATCC Accession No. CRL-12223).

46. The CHO cell of claim 43 designated C-rGalR2-79
    (ATCC Accession No. _____).

47. An insect cell comprising the vector of claim 31.

48. An insect cell of claim 47, wherein the insect cell
    is an Sf9 cell.

49. An insect cell of claim 47, wherein the insect cell
    is an Sf21 cell.

50. A membrane preparation isolated from the cell of
    either of claims 39 or 47.

51. A nucleic acid probe comprising at least 15
    nucleotides, which probe specifically hybridizes
    with a nucleic acid encoding a GALR2 receptor,
    wherein the probe has a unique sequence
    corresponding to a sequence present within one of
    the two strands of the nucleic acid encoding the
    GALR2 receptor contained in plasmid K985.

52. A nucleic acid probe comprising at least 15
    nucleotides, which probe specifically hybridizes
    with a nucleic acid encoding a GALR2 receptor,
    wherein the probe has a unique sequence

corresponding to a sequence present within one of
the two strands of the nucleic acid encoding the
GALR2 receptor contained in plasmid K1045.

5   53.   A nucleic acid probe comprising at least 15
            nucleotides, which probe specifically hybridizes
            with a nucleic acid encoding a GALR2 receptor,
            wherein the probe has a unique sequence
            corresponding to a sequence present within (a) the
10   nucleic acid sequence shown in Figure 1 or (b) the
            reverse complement of the nucleic acid sequence
            shown in Figure 1.

      54.   A nucleic acid probe comprising at least 15
15   nucleotides, which probe specifically hybridizes
            with a nucleic acid encoding a GALR2 receptor,
            wherein the probe has a unique sequence
            corresponding to a sequence present within one of
            the two strands of the nucleic acid encoding the
20   GALR2 receptor contained in plasmid BO29.

      55.   A nucleic acid probe comprising at least 15
            nucleotides, which probe specifically hybridizes
            with a nucleic acid encoding a GALR2 receptor,
25   wherein the probe has a unique sequence
            corresponding to a sequence present within one of
            .the two strands of the nucleic acid encoding the
            GALR2 receptor contained in plasmid BO39.

30   56.   A nucleic acid probe comprising at least 15
            nucleotides, which probe specifically hybridizes
            with a nucleic acid encoding a GALR2 receptor,
            wherein the probe has a unique sequence
            corresponding to a sequence present within (a) the
35   nucleic acid sequence shown in Figure 10 or (b) the

reverse complement of the nucleic acid sequence shown in Figure 10.

57.   The nucleic acid probe of any one of claims 51, 52, 53, 54, 55 or 56, wherein the nucleic acid is DNA.

58.   The nucleic acid probe of any one of claims 51, 52, 53, 54, 55 or 56 wherein the nucleic acid is RNA.

59.   A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a unique fragment of the sequence of a nucleic acid molecule encoding a GALR2 receptor.

60.   A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to the antisense sequence of a unique fragment of the sequence of a nucleic acid molecule encoding a GALR2 receptor.

61.   An antisense oligonucleotide having a sequence capable of specifically hybridizing to the mRNA of claim 5, so as to prevent translation of the mRNA.

62.   An antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA of claim 4.

63.   An antisense oligonucleotide of either of claims 61 or 62, wherein the oligonucleotide comprises chemically modified nucleotides or nucleotide analogues.

64.   An antibody capable of binding to a GALR2 receptor

encoded by the nucleic acid of claim 1.

65.   The antibody of claim 64, wherein the GALR2 receptor
       is a human GALR2 receptor.

66.   An antibody capable of competitively inhibiting the
       binding of the antibody of claim 64 to a GALR2
       receptor.

67.   An antibody of claim 64, wherein the antibody is a
       monoclonal antibody.

68.   A monoclonal antibody of claim 67 directed to an
       epitope of a GALR2 receptor present on the surface
       of a GALR2 receptor expressing cell.

69..   A pharmaceutical composition comprising an amount of
       the oligonucleotide of claim 61 capable of passing
       through a cell membrane effective to reduce
       expression of a GALR2 receptor and a
       pharmaceutically acceptable carrier capable of
       passing through a cell membrane.

70.   A pharmaceutical composition of claim 69, wherein
       the oligonucleotide is coupled to a substance which
       inactivates mRNA.

71.   A pharmaceutical composition of claim 70, wherein
       the substance which inactivates mRNA is a ribozyme.

72.   A pharmaceutical composition of claim 69, wherein
       the pharmaceutically acceptable carrier comprises a
       structure which binds to a receptor on a cell
       capable of being taken up by the cells after binding
       to the structure.

73. A pharmaceutical composition of claim 72, wherein the pharmaceutically acceptable carrier is capable of binding to a receptor which is specific for a selected cell type.

74. A pharmaceutical composition which comprises an amount of the antibody of claim 64 effective to block binding of a ligand to the GALR2 receptor and a pharmaceutically acceptable carrier.

75. A transgenic nonhuman mammal expressing DNA encoding a GALR2 receptor of claim 1.

76. A transgenic nonhuman mammal comprising a homologous recombination knockout of the native GALR2 receptor.

77. A transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a GALR2 receptor of claim 1 so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a GALR2 receptor and which hybridizes to mRNA encoding a GALR2 receptor, thereby reducing its translation.

78. The transgenic nonhuman mammal of claim 75 or 76, wherein the DNA encoding a GALR2 receptor additionally comprises an inducible promoter.

79. The transgenic nonhuman mammal of claim 75 or 77, wherein the DNA encoding a GALR2 receptor additionally comprises tissue specific regulatory elements.

80. A transgenic nonhuman mammal of any one of claims 75, 76 or 77, wherein the transgenic nonhuman mammal

is a mouse.

81.   A process for identifying a chemical compound which
       specifically binds to a GALR2 receptor which
       comprises contacting cells containing DNA encoding
       and expressing on their cell surface the GALR2
       receptor, wherein such cells do not normally express
       the GALR2 receptor, with the compound under
       conditions suitable for binding, and detecting
       specific binding of the chemical compound to the
       GALR2 receptor.

82.   A process for identifying a chemical compound which
       specifically binds to a GALR2 receptor which
       comprises contacting a membrane fraction from a cell
       extract of cells containing DNA encoding and
       expressing on their cell surface the GALR2 receptor,
       wherein such cells do not normally express the GALR2
       receptor, with the compound under conditions
       suitable for binding, and detecting specific binding
       of the chemical compound to the GALR2 receptor.

83.   The process of claim 81 or 82, wherein the GALR2
       receptor is a mammalian GALR2 receptor.

84.   The process of claim 81 or 82, wherein the GALR2
       receptor has substantially the same amino acid
       sequence as encoded by the plasmid K985 (ATCC
       Accession No. 97426).

85.   The process of claim 81 or 82, wherein the GALR2
       receptor has substantially the same amino acid
       sequence as that shown in Figure 2 (Seq. ID No. 8).

86.   The process of claim 81 or 82, wherein the GALR2

receptor has substantially the same amino acid
sequence as encoded by the plasmid BO29 (ATCC
Accession No. 97735).

5   87. The process of claim 81 or 82, wherein the GALR2
          receptor has substantially the same amino acid
          sequence as that shown in Figure 11 (Seq. ID No.
          30).

10   88. The method of any one of claims 81, 82, 83, 84, 85,
          86 or 87, wherein the compound is not previously
          known to bind to a GALR2 receptor.

     89. A compound determined by the method of claim 88.
15

     90. A   process   for   determining   whether   a   chemical
          compound is a GALR2 receptor agonist which comprises
          contacting cells transfected with and expressing DNA
          encoding the GALR2 receptor with the compound under
20   conditions permitting the activation of the GALR2
          receptor,   and   detecting   an   increase   in   GALR2
          receptor   activity,   so   as   to   thereby   determine
          whether the compound is a GALR2 receptor agonist.

25   91. A   process   for   determining   whether   a   chemical
          compound is a GALR2 receptor agonist which comprises
          preparing a cell extract from cells transfected with
          and   expressing   DNA   encoding   the   GALR2   receptor,
          isolating a membrane fraction from the cell extract,
30   contacting the membrane fraction with the compound
          under conditions permitting the activation of the
          GALR2 receptor, and detecting an increase in GALR2
          receptor   activity,   so   as   to   thereby   determine
          whether the compound is a GALR2 receptor agonist.

35

92. The process of claim 90 or 91, wherein the GALR2 receptor is a mammalian GALR2 receptor.

93. The process of claim 90 or 91, wherein the GALR2 receptor has substantially the same amino acid sequence as encoded by the plasmid K985 (ATCC Accession No. 97426).

94. The process of claim 90 or 91, wherein the GALR2 receptor has substantially the same amino acid sequence as that shown in Figure 2 (Seq. ID No. 8).

95. The process of claim 90 or 91, wherein the GALR2 receptor has substantially the same amino acid sequence as encoded by the plasmid BO29 (ATCC Accession No. 97735).

96. The process of claim 90 or 91, wherein the GALR2 receptor has substantially the same amino acid sequence as that shown in Figure 11 (Seq. ID No. 30).

97. A process for determining whether a chemical compound is a GALR2 receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding the GALR2 receptor with the compound in the presence of a known GALR2 receptor agonist, under conditions permitting the activation of the GALR2 receptor, and detecting a decrease in GALR2 receptor activity, so as to thereby determine whether the compound is a GALR2 receptor antagonist.

98. A process for determining whether a chemical compound is a GALR2 receptor antagonist which comprises preparing a cell extract from cells

transfected with and expressing DNA encoding the
GALR2 receptor, isolating a membrane fraction from
the cell extract, contacting the membrane fraction
with the ligand in the presence of a known GALR2
receptor agonist, under conditions permitting the
activation of the GALR2 receptor, and detecting a
decrease in GALR2 receptor activity, so as to
thereby determine whether the compound is a GALR2
receptor antagonist.

99.   The process of claim 97 or 98, wherein the GALR2
       receptor is a mammalian GALR2 receptor.

100.   The process of claim 97 or 98, wherein the GALR2
       receptor has substantially the same amino acid
       sequence as encoded by the plasmid K985 (ATCC
       Accession No. 97426).

101.   The process of claim 97 or 98, wherein the GALR2
       receptor has substantially the same amino acid
       sequence as that shown in Figure 2 (Seq. ID No. 8).

102.   The process of claim 97 or 98, wherein the GALR2
       receptor has substantially the same amino acid
       sequence as encoded by the plasmid BO29 (ATCC
       Accession No. 97735).

103.   The process of claim 97 or 98, wherein the GALR2
       receptor has substantially the same amino acid
       sequence as that shown in Figure 11 (Seq. ID No.
       30).

104.   The process of any one of claims 81, 82, 83, 84, 85,
       86, 87, 88, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99,
       100, 101, 102 or 103, wherein the cell is an insect

cell.

105. The process of any one of claims 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102 or 103, wherein the cell is a mammalian cell.

106. The process of claim 105, wherein the cell is nonneuronal in origin.

107. The process of claim 106, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell.

108. The process of claim 106, wherein the nonneuronal cell is the LM(tk-) cell designated L-rGALR2-8 (ATCC Accession No. CRL-12074).

109. The process of claim 106, wherein the nonneuronal cell is the LM(tk-) cell designated L-rGALR2I-4 (ATCC Accession No. CRL-12223).

110. The process of claim 106, wherein the nonneuronal cell is the CHO cell designated C-rGalR2-79 (ATCC Accession No. _____).

111. The process of claim 105 wherein the compound is not previously known to bind to a GALR2 receptor.

112. A compound determined by the process of claim 111.

113. A pharmaceutical composition which comprises an amount of a GALR2 receptor agonist determined by the process of claim 90 or 91 effective to increase activity of a GALR2 receptor and a pharmaceutically

acceptable carrier.

114. A pharmaceutical composition of claim 113, wherein
     the GALR2 receptor agonist is not previously known.

115. A pharmaceutical composition which comprises an
     amount of a GALR2 receptor antagonist determined by
     the process of claim 97 or 98 effective to reduce
     activity of a GALR2 receptor and a pharmaceutically
     acceptable carrier.

116. A pharmaceutical composition of claim 115, wherein
     the GALR2 receptor antagonist is not previously
     known.

117. A process involving competitive binding for
     identifying a chemical compound which specifically
     binds to a GALR2 receptor which comprises separately
     contacting cells expressing on their cell surface
     the GALR2 receptor, wherein such cells do not
     normally express the GALR2 receptor, with both the
     chemical compound and a second chemical compound
     known to bind to the receptor, and with only the
     second chemical compound, under conditions suitable
     for binding of both compounds, and detecting
     specific binding of the chemical compound to the
     GALR2 receptor, a decrease in the binding of the
     second chemical compound to the GALR2 receptor in
     the presence of the chemical compound indicating
     that the chemical compound binds to the GALR2
     receptor.

118. A process involving competitive binding for
     identifying a chemical compound which specifically
     binds to a human GALR2 receptor which comprises

separately contacting a membrane fraction from a
cell extract of cells expressing on their cell
surface the GALR2 receptor, wherein such cells do
not normally express the GALR2 receptor, with both
the chemical compound and a second chemical compound
known to bind to the receptor, and with only the
second chemical compound, under conditions suitable
for binding of both compounds, and detecting
specific binding of the chemical compound to the
GALR2 receptor, a decrease in the binding of the
second chemical compound to the GALR2 receptor in
the presence of the chemical compound indicating
that the chemical compound binds to the GALR2
receptor.

119. A process for determining whether a chemical
compound specifically binds to and activates a GALR2
receptor, which comprises contacting cells producing
a second messenger response and expressing on their
cell surface the GALR2 receptor, wherein such cells
do not normally express the GALR2 receptor, with the
chemical compound under conditions suitable for
activation of the GALR2 receptor, and measuring the
second messenger response in the presence and in the
absence of the chemical compound, a change in the
second messenger response in the presence of the
chemical compound indicating that the compound
activates the GALR2 receptor.

120. A process for determining whether a chemical
compound specifically binds to and activates a
GALR2 receptor, which comprises contacting a
membrane fraction isolated from a cell extract of
cells producing a second messenger response and
expressing on their cell surface the GALR2 receptor,

wherein such cells do not normally express the GALR2 receptor, with the chemical compound under conditions suitable for activation of the GALR2 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the GALR2 receptor.

121. A process for determining whether a chemical compound specifically binds to and inhibits activation of a GALR2 receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the GALR2 receptor, wherein such cells do not normally express the GALR2 receptor, with both the chemical compound and a second chemical compound known to activate the GALR2 receptor, and with only the second chemical compound, under conditions suitable for activation of the GALR2 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the GALR2 receptor.

122. A process for determining whether a chemical compound specifically binds to and inhibits activation of a GALR2 receptor, which comprises separately contacting a membrane fraction from a

cell extract of cells producing a second messenger response and expressing on their cell surface the GALR2 receptor, wherein such cells do not normally express the GALR2 receptor, with both the chemical compound and a second chemical compound known to activate the GALR2 receptor, and with only the second chemical compound, under conditions suitable for activation of the GALR2 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the GALR2 receptor.

123. The process of claim 119 or 120, wherein the second messenger response comprises arachidonic acid release and the change in second messenger response is an increase in arachidonic acid levels.

124. The process of claim 119 or 120, wherein the second messenger response comprises intracellular calcium levels and the change in second messenger response is an increase in intracellular calcium levels.

125. The process of claim 119 or 120, wherein the second messenger response comprises inositol phospholipid hydrolysis and the change in second messenger response is an increase in inositol phospholipid hydrolysis.

126. The process of claim 121 or 122, wherein the second

- 184 -

messenger response comprises arachidonic acid
release and the change in second messenger response
is a smaller increase in the level of arachidonic
acid in the presence of both the chemical compound
5   and the second chemical compound than in the
presence of only the second chemical compound.

127. The process of claim 121 or 122, wherein the second
messenger response comprises intracellular calcium
10   levels, and the change in second messenger response
is a smaller increase in the intracellular calcium
levels in the presence of both the chemical compound
and the second chemical compound than in the
presence of only the second chemical compound.

15

128. The process of claim 121 or 122, wherein the second
messenger response comprises inositol phospholipid
hydrolysis, and the change in second messenger
response is a smaller increase in inositol
20   phospholipid hydrolysis in the presence of both the
chemical compound and the second chemical compound
than in the presence of only the second chemical
compound.

25   129. A process of any one of claims 117, 118, 119, 120,
121, 122, 123, 124, 125, 126, 127 or 128, wherein
the GALR2 receptor is a mammalian GALR2 receptor.

130. The process of claim 129, wherein the GALR2 receptor
30   has substantially the same amino acid sequence as
encoded by the plasmid K985 ATCC Accession No.
97426).

131. The process of claim 129, wherein the GALR2 receptor
35   has substantially the same amino acid sequence as

that shown in Figure 2 (Seq. ID No. 8).

132. The process of claim 129, wherein the GALR2 receptor
     has substantially the same amino acid sequence as
     encoded by the plasmid BO29 (ATCC Accession No.
     97735).

133. The process of claim 129, wherein the GALR2 receptor
     has substantially the same amino acid sequence as
     that shown in Figure 11 (Seq. ID No. 30).

134. The process of any one of claims 117, 118, 119, 120,
     121, 122, 123, 124, 125, 126, 127, 128, 129, 130,
     131, 132 or 133, wherein the cell is an insect cell.

135. The process of any one of claims 117, 118, 119, 120,
     121, 122, 123, 124, 125, 126, 127, 128, 129, 130,
     131, 132 or 133, wherein the cell is a mammalian
     cell.

136. The process of claim 135, wherein the mammalian cell
     is nonneuronal in origin.

137. The process of claim 136, wherein the nonneuronal
     cell is a COS-7 cell, 293 human embryonic kidney
     cell, NIH-3T3 cell or LM(tk-) cell.

138. The process of claim 136, wherein the nonneuronal
     cell is the LM(tk-) cell designated L-rGALR2-8 (ATCC
     Accession No. CRL-12074).

139. The process of claim 136, wherein the nonneuronal
     cell is the LM(tk-) cell designated L-rGALR2I-4
     (ATCC Accession No. CRL-12223).

140. The process of claim 136, wherein the nonneuronal
     cell is the CHO cell designated C-rGalR2-79 (ATCC
     Accession No. _____).

141. The process of claim 135, wherein the compound is
     not previously known to bind to a GALR2 receptor.

142. A compound determined by the process of claim 141.

143. A pharmaceutical composition which comprises an
     amount of a GALR2 receptor agonist determined by the
     process of any one of claims 119, 120, 123, 124, or
     125 effective to increase activity of a GALR2
     receptor and a pharmaceutically acceptable carrier.

144. A pharmaceutical composition of claim 143, wherein
     the GALR2 receptor agonist is not previously known.

145. A pharmaceutical composition which comprises an
     amount of a GALR2 receptor antagonist determined by
     the process of any one of claims 121, 122, 126, 127
     or 128 effective to reduce activity of a GALR2
     receptor and a pharmaceutically acceptable carrier.

146. A pharmaceutical composition of claim 145, wherein
     the GALR2 receptor antagonist is not previously
     known.

147. A method of screening a plurality of chemical
     compounds not known to bind to a GALR2 receptor to
     identify a compound which specifically binds to the
     GALR2 receptor, which comprises

     (a) contacting cells transfected with and
         expressing DNA encoding the GALR2 receptor with

a compound known to bind specifically to the
GALR2 receptor;

(b) contacting the preparation of step (a) with the
plurality of compounds not known to bind
specifically to the GALR2 receptor, under
conditions permitting binding of compounds
known to bind the GALR2 receptor;

(c) determining whether the binding of the compound
known to bind to the GALR2 receptor is reduced
in the presence of the compounds, relative to
the binding of the compound in the absence of
the plurality of compounds; and if so

(d) separately determining the binding to the GALR2
receptor of each compound included in the
plurality of compounds, so as to thereby
identify the compound which specifically binds
to the GALR2 receptor.

148. A method of screening a plurality of chemical
compounds not known to bind to a GALR2 receptor to
identify a compound which specifically binds to the
GALR2 receptor, which comprises

(a) preparing a cell extract from cells transfected
with and expressing DNA encoding the GALR2
receptor, isolating a membrane fraction from
the cell extract, contacting the membrane
fraction with a compound known to bind
specifically to the GALR2 receptor;

(b) contacting the preparation of step (a) with the
plurality of compounds not known to bind

- 188 -

specifically to the GALR2 receptor, under
conditions permitting binding of compounds
known to bind the GALR2 receptor;

5   (c)   determining whether the binding of the compound
known to bind to the GALR2 receptor is reduced
in the presence of the compounds, relative to
the binding of the compound in the absence of
the plurality of compounds; and if so

10

(d)   separately determining the binding to the GALR2
receptor of each compound included in the
plurality of compounds, so as to thereby
identify the compound which specifically binds

15   to the GALR2 receptor.

149. A method of claim 147 or 148, wherein the GALR2
receptor is a mammalian GALR2 receptor.

20   150. A method of screening a plurality of chemical
compounds not known to activate a GALR2 receptor to
identify a compound which activates the GALR2
receptor which comprises

25   (a)   contacting cells transfected with and
expressing the GALR2 receptor with the
plurality of compounds not known to activate
the GALR2 receptor, under conditions permitting
activation of the GALR2 receptor;

30

(b)   determining whether the activity of the GALR2
receptor is increased in the presence of the
compounds; and if so

35   (c)   separately determining whether the activation

of the GALR2 receptor is increased by each
compound included in the plurality of
compounds, so as to thereby identify the
compound which activates the GALR2 receptor.

151. A method of screening a plurality of chemical
compounds not known to activate a GALR2 receptor to
identify a compound which activates the GALR2
receptor which comprises

(a)   preparing a cell extract from cells transfected
with and expressing DNA encoding the GALR2
receptor, isolating a membrane fraction from
the cell extract, contacting the membrane
fraction with the plurality of compounds not
known to activate the GALR2 receptor, under
conditions permitting activation of the GALR2
receptor;

(b)   determining whether the activity of the GALR2
receptor is increased in the presence of the
compounds; and if so

(c)   separately determining whether the activation
of the GALR2 receptor is increased by each
compound included in the plurality of
compounds, so as to thereby identify the
compound which activates the GALR2 receptor.

152. A method of claim 150 or 151, wherein the GALR2
receptor is a mammalian GALR2 receptor.

153. A method of screening a plurality of chemical
compounds not known to inhibit the activation of a
GALR2 receptor to identify a compound which inhibits

the activation of the GALR2 receptor, which comprises

(a) contacting cells transfected with and expressing the GALR2 receptor with the plurality of compounds in the presence of a known GALR2 receptor agonist, under conditions permitting activation of the GALR2 receptor;

(b) determining whether the activation of the GALR2 receptor is reduced in the presence of the plurality of compounds, relative to the activation of the GALR2 receptor in the absence of the plurality of compounds; and if so

(c) separately determining the inhibition of activation of the GALR2 receptor for each compound included in the plurality of compounds, so as to thereby identify the compound which inhibits the activation of the GALR2 receptor.

154. A method of screening a plurality of chemical compounds not known to inhibit the activation of a GALR2 receptor to identify a compound which inhibits the activation of the GALR2 receptor, which comprises

(a) preparing a cell extract from cells transfected with and expressing DNA encoding the GALR2 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the plurality of compounds in the presence of a known GALR2 receptor agonist, under conditions permitting activation of the

GALR2 receptor;

(b)   determining whether the activation of the GALR2
        receptor is reduced in the presence of the
        plurality of compounds, relative to the
        activation of the GALR2 receptor in the absence
        of the plurality of compounds; and if so

(c)   separately determining the inhibition of
        activation of the GALR2 receptor for each
        compound included in the plurality of
        compounds, so as to thereby identify the
        compound which inhibits the activation of the
        GALR2 receptor.

155.   A method of any one of claims 90, 91, 97, 98, 150,
       151, 153, or 154, wherein activation of the GALR2
       receptor is determined by a second messenger assay.

156.   The method of claim 155, wherein the second
       messenger is cyclic AMP, intracellular calcium, or
       an inositol phospholipid.

157.   A method of claim 153 or 154, wherein the GALR2
       receptor is a mammalian GALR2 receptor.

158.   A method of any one of claims 147, 148, 150, 151,
       153, or 154, wherein the cell is a mammalian cell.

159.   A method of claim 158, wherein the mammalian cell is
       non-neuronal in origin.

160.   The method of claim 159, wherein the non-neuronal
       cell is a COS-7 cell, a 293 human embryonic kidney
       cell, a LM(tk-) cell or an NIH-3T3 cell.

161. A pharmaceutical composition comprising a compound
     identified by the method of claim 150 and a
     pharmaceutically acceptable carrier.

162. A pharmaceutical composition comprising a compound
     identified by the method of claim 153 and a
     pharmaceutically acceptable carrier.

163. A method of detecting expression of a GALR2 receptor
     by detecting the presence of mRNA coding for the
     GALR2 receptor which comprises obtaining total mRNA
     from the cell and contacting the mRNA so obtained
     with the nucleic acid probe of any one of claims
     51, 52, 53, 54, 55, 56 or 60 under hybridizing
     conditions, detecting the presence of mRNA
     hybridized to the probe, and thereby detecting the
     expression of the GALR2 receptor by the cell.

164. A method of treating an abnormality in a subject,
     wherein the abnormality is alleviated by the
     inhibition of a GALR2 receptor which comprises
     administering to a subject an effective amount of
     the pharmaceutical composition of any one of claims
     115, 116, or 162 effective to decrease the activity
     of the GALR2 receptor in the subject, thereby
     treating the abnormality in the subject.

165. The method of claim 164, wherein the abnormality is
     obesity or bulimia.

166. A method of treating an abnormality in a subject
     wherein the abnormality is alleviated by the
     activation of a GALR2 receptor which comprises
     administering to a subject an effective amount of
     the pharmaceutical composition of any one of claims

- 193 -

113, 114, or 161 effective to activate the GALR2 receptor in the subject.

167. The method of claim 166, wherein the abnormal condition is anorexia.

168. The method of claim 164 or 166, wherein the compound binds selectively to a GALR2 receptor.

169. The method of claim 168, wherein the compound binds to the GALR2 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to a GALR1 receptor.

170. The method of claim 168, wherein the compound binds to the GALR2 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to a GALR3 receptor.

171. A method of detecting the presence of a GALR2 receptor on the surface of a cell which comprises contacting the cell with the antibody of claim 64 under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a GALR2 receptor on the surface of the cell.

172. A method of determining the physiological effects of varying levels of activity of GALR2 receptors which comprises producing a transgenic nonhuman mammal of claim 78 whose levels of GALR2 receptor activity are varied by use of an inducible promoter which regulates GALR2 receptor expression.

- 194 -

173. A method of determining the physiological effects of varying levels of activity of GALR2 receptors which comprises producing a panel of transgenic nonhuman mammals of claim 78 each expressing a different amount of GALR2 receptor.

174. A method for identifying an antagonist capable of alleviating an abnormality wherein the abnormality is alleviated by decreasing the activity of a GALR2 receptor comprising administering a compound to the transgenic nonhuman mammal of any one of claims 75, 78, 79, or 80, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overactivity of a GALR2 receptor, the alleviation of the abnormality identifying the compound as an antagonist.

175. An antagonist identified by the method of claim 174.

176. A pharmaceutical composition comprising an antagonist identified by the method of claim 174 and a pharmaceutically acceptable carrier.

177. A method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a GALR2 receptor which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 176, thereby treating the abnormality.

178. A method for identifying an agonist capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a GALR2 receptor comprising administering a

compound to the transgenic nonhuman mammal of any
one of claims 75, 78, 79, or 80, and determining
whether the compound alleviates the physical and
behavioral abnormalities displayed by the transgenic
nonhuman mammal, the alleviation of the abnormality
identifying the compound as an agonist.

179. An agonist identified by the method of claim 178.

180. A pharmaceutical composition comprising an agonist
identified by the method of claim 178 and a
pharmaceutically acceptable carrier.

181. A method for treating an abnormality in a subject
wherein the abnormality is alleviated by increasing
the activity of a GALR2 receptor which comprises
administering to a subject an effective amount of
the pharmaceutical composition of claim 180, thereby
treating the abnormality.

182. A method for diagnosing a predisposition to a
disorder associated with the activity of a specific
human GALR2 receptor allele which comprises:

a. obtaining DNA of subjects suffering from the
    disorder;

b. performing a restriction digest of the DNA with
    a panel of restriction enzymes;

c. electrophoretically separating the resulting
    DNA fragments on a sizing gel;

d. contacting the resulting gel with a nucleic
    acid probe capable of specifically hybridizing

with a unique sequence included within the sequence of a nucleic acid molecule encoding a human GALR2 receptor and labelled with a detectable marker;

e. detecting labelled bands which have hybridized to the DNA encoding a human GALR2 receptor of claim 9 labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder;

f. preparing DNA obtained for diagnosis by steps a-e; and

g. comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

183. The method of claim 182, wherein a disorder associated with the activity of a specific human GALR2 receptor allele is diagnosed.

184. A method of preparing the purified GALR2 receptor of claim 26, which comprises:

a. inducing cells to express GALR2 receptor;

b. recovering the receptor from the induced cells; and

c. purifying the receptor so recovered.

185. A method of preparing the purified GALR2 receptor of claim 26, which comprises:

5   a.   inserting nucleic acid encoding the GALR2 receptor in a suitable vector;

       b.   introducing the resulting vector in a suitable host cell;

10

       c.   placing the resulting cell in suitable condition permitting the production of the isolated GALR2 receptor;

15   d.   recovering the receptor produced by the resulting cell; and

       e.   purifying the receptor so recovered.

20   186. A method of modifying feeding behavior of a subject which comprises administering to the subject an amount of a compound which is a GALR2 receptor agonist or antagonist effective to increase or decrease the consumption of food by the subject so
25   as to thereby modify feeding behavior of the subject.

187. The method of claim 186, wherein the compound is a GALR2 receptor antagonist and the amount is
30   effective to decrease the consumption of food by the subject.

188. The method of claim 186 or 187, wherein the compound is administered in combination with food.

35

189. The method of claim 186, wherein the compound is a
GALR2 receptor agonist and the amount is effective
to increase the consumption of food by the subject.

5   190. The method of claim 186 or 189, wherein the compound
is administered in combination with food.

191. The method of claim 186 or 189, wherein the compound
binds selectively to a GALR2 receptor.

10

192. The method of claim 191, wherein the compound binds
to the GALR2 receptor with an affinity greater than
ten-fold higher than the affinity with which the
compound binds to a GALR1 receptor.

15

193. The method of claim 191, wherein the compound binds
to the GALR2 receptor with an affinity greater than
ten-fold higher than the affinity with which the
compound binds to a GALR3 receptor.

20

194. The method of claim 191, wherein the compound binds
to the GALR2 receptor with an affinity greater than
one hundred-fold higher than the affinity with which
the compound binds to a GALR1 receptor.

25

195. The method of claim 191, wherein the compound binds
to the GALR2 receptor with an affinity greater than
one hundred-fold higher than the affinity with which
the compound binds to a GALR3 receptor.

30

196. The method of claim 186, wherein the subject is a
vertebrate, a mammal, a human or a canine.

197. A method for determining whether a compound is a
35   GALR2 antagonist which comprises:

(a)   administering to an animal a GALR2 agonist and
        measuring the amount of food intake in the
        animal;

5   (b)   administering to a second animal both the GALR2
                agonist and the compound, and measuring the
                amount of food intake in the second animal; and

        (c)   determining whether the amount of food intake
10   is reduced in the presence of the compound
                relative to the amount of food intake in the
                absence of the compound, so as to thereby
                determine whether the compound is a GALR2
                antagonist.

15
        198. A method of screening a plurality of compounds to
             identify a compound which is a GALR2 antagonist
             which comrises:

20   (a)   administering to an animal a GALR2 agonist and
                measuring the amount of food intake in the
                animal;

        (b)   administering to a second animal the GALR2
25   agonist and at least one compound of the
                plurality of compounds and measuring the amount
                of food intake in the animal;

        (c)   determining whether the amount of food intake
30   is reduced in the presence of at least one
                compound of the plurality relative to the
                amount of food intake in the absence of at
                least one compound of the plurality, and if so;

35   (d)   separately determining whether each compound is

- 200 -

a GALR2 antagonist according to the method of
claim 132, so as to thereby identify a compound
which is a GALR2 antagonist.

5   199. The method of claim 197 or 198, wherein the GALR2
            agonist is [D-Trp]$_2$-galanin $_{(1-29)}$.

       200. The method of either of claims 197 or 198 wherein
            the animal is a non-human mammal.

10

       201. The method of claim 200, wherein the mammal is a
            rodent.

       202. A process of claim 81 or 82, which further comprises
15   determining whether the compound selectively binds
            to the GALR2 receptor relative to another galanin
            receptor.

       203. The process of claim 202, wherein the determination
20   whether the compound selectively binds to the GALR2
            receptor comprises:

            (a) determining the binding affinity of the
                 compound for the GALR2 receptor and for such
25   other galanin receptor; and

            (b) comparing the binding affinities so determined,
                 the presence of a higher binding affinity for
                 the GALR2 receptor than for such other galanin
30   receptor inicating that the compound
                 selectively binds to the GALR2 receptor.

       204. A process of claim 202, wherein such other galanin
            receptor is a GALR1 receptor.

35

205. A process of claim 202, wherein such other galanin receptor is a GALR3 receptor.

206. A method of decreasing feeding behavior of a subject which comprises administering a compound which is a GALR2 receptor antagonist and a compound which is a Y5 receptor antagonist, the amount of such antagonists being effective to decrease the feeding behavior of the subject.

207. The method of claim 206, wherein the GALR2 antagonist and the Y5 antagonist are administered in combination.

208. The method of claim 206, wherein the GALR2 antagonist and the Y5 antagonist are administered once.

209. The method of claim 206, wherein the GALR2 antagonist and the Y5 antagonist are administered separately.

210. The method of claim 209, wherein the GALR2 antagonist and the Y5 antagonist are administered once.

211. The method of claim 209, wherein the galanin receptor antagonist is administered for about 1 week to 2 weeks.

212. The method of claim 209, wherein the Y5 receptor antagonist is administered for about 1 week to 2 weeks.

213. The method of claim 209, wherein the GALR2

antagonist and the Y5 antagonist are administered
alternately.

214. The method of claim 213, wherein the GALR2
antagonist and the Y5 antagonist are administered
repeatedly.

215. A method of claim 213 or 214, wherein the galanin
receptor antagonist is administered for about 1 week
to 2 weeks.

216. A method of claim 213 or 214, wherein the Y5
receptor antagonist is administered for about 1 week
to 2 weeks.

217. A method of any one of claims 206, 207, 208, or 209,
wherein the compound is administered in a
pharmaceutical composition comprising a sustained
release formulation.

218. A method of decreasing nociception in a subject
which comprises administering to the subject an
amount of a compound which is a GALR2 receptor
agonist effective to decrease nociception in the
subject.

219. A method of treating pain in a subject which
comprises administering to the subject an amount of
a compound which is a GALR2 receptor agonist
effective to treat pain in the subject.

220. A method of treating Alzheimer's disease in a
subject which comprises administering to the subject
an amount of a compound which is a GALR2 receptor
antagonist effective to treat Alzheimer's disease in

the subject.

FIGURE 1

```
   1  CAAGACCCGGACAGCTGCGGGAGCGGCGTCCACTTTGGTGATACCATGAATGGCTCCGGC      60
  61  AGCCAGGGCGCGGGAGAACAGAGCCAGGAAGGCGGTAGCGGCGGCTGGCAGCCTGAGGCG     120
 121  GTCCTTGTACCCCTATTTTCGCGCGGCTCATCTTCCTCGTGGGCACCGTGGGCAACGCGCTG   180
 181  GTGCTGGCGGTGCTGCTGCCGACCTGTGTTTCATCTCCTGTGCTGCTCGCTGGGTGTTCGG    240
 241  AACCTGGGCGTGGCCGACCTGGGTGTTCGGTTTCATCCTGTGCTGCTCTGCGTGCCGTGCTC   300
 301  TACACCCTGGACGACTGGGTGTTCGGTTTCATCCTGTGCTGCTCTGCAAGGCTGTTCATTTCCTCATC   360
 361  TTTCTCACTATGCACGCCAGCAGCTTCACGCTCCCGAGCGTCTCCCGTCCCGCGTTCCCTGGACAGGTATCTG   420
 421  GCCATCCGCTACCCGCTGCCACTCCCGAGAGTTGCGCACACCTGCGAAACGCGCTGGCCGCC   480
 481  ATCGGGCTCATCTGGGGCTAGCACTGCTCTTCTCCGGGCCCCTACCTGAGCTGCACGTCGA   540
 541  CAGTCGCAGCTGGCCAACCTGCACCCTTCGTCTTTAGCTACCTGCCAGTGCTCCTCAGTCTG    600
 601  GCCATGGACCTTCGCGCTACCCTGCGCTACCTCTGGCACAGTGCTGCCAGTCGACGTCGA    660
 661  ACCTATGCGCGTACCCCGCCAAAGCGCAAGGTGACACGGATGATCATCGTGGCGGTCTCA    720
 721  GGTTCCCAGCGCGTACCCCGCCAAAGCGCAAGGTGACACGGATGATCATCGTGGCGGTGGTTTC   780
 781  TGCCTCTGTTGGATGCCCACTTACGCGCTTATCCTCGTGGTGTTGGTTTGTGCGCTTCCCG    840
 841  CTCACGCGTGCCACTCCATCGTTTACGCGCTGCGCCCCTAGTTTCACACCTAGTTCCTATGCCAACTCC     900
 901  TGTGTCAACCCCATCGTTTACGCGCTGCGCCCCTGCGCCCCGAGTGCCGGCATTTCCGTAAAGGTTTCCGCAAA   960
 961  ATCTGCGCGGGCCTGGGAACCATAGTGGCAGCATGCTGGGCCGAGTTCGGGCCGAGCTTCGGGCCGAGCATCCTG  1020
1021  GCGCCTGGGAACCATAGTGGCAGCATGCTGGGCCGAGTTCGGGCCGAGCATCCTG          1080
1081  AGCGAGGCAGCCGGCGGCCCCTTGTCTGCCACCACCCCGCCACTCCCAACTGACAGCCTCGAGT  1140
1141  AGAACCCTGGATCCGGCTTGTTAAAGGACCAAAGGGCATCTAACAGCTTCTAG            1193
```

FIGURE 2

```
  1  MNGSGSQGAE NTSQEGGSGG                          20
 21  WQPEAVLVPL FFALIFLVGT                          40
 41  VGNHLVLAVL LFGGQAVSTT                          60
 61  NLFTHNLGVA DRCEHLCCVP                          80
 81  FQALHYTLDD WLFGSLLCKA                         100
101  VHFYLFLTMH AVSFTLAAVS                         120
121  LDRAAAHRYP LSSRELRTPR                         140
141  NALYRIGLIW GHALLFSGPY                         160
161  LSYRRQSQLA NLTVCHPAWS                         180
181  APRSLAMDRC TLVFSHLLPV                         200
201  LVLGSTYART LFYLWRTVDP                         220
221  VTALFGSQWA KRKVTRMIII                         240
241  VAVFPCLCAM PRHALILCVW                         260
261  FGRNSLTRPT YHLRILSHLV                         280
281  SYARKCVNGI VAALVSKHFR                         300
301  KGFILICANL LYPAPRRASG                         320
321  RVSQVAPGAH SRSMLEQEPT                         340
341  DLTSSSEADG PGVPPPALPN                         360
361  CTARTLDPAC                                    372
```
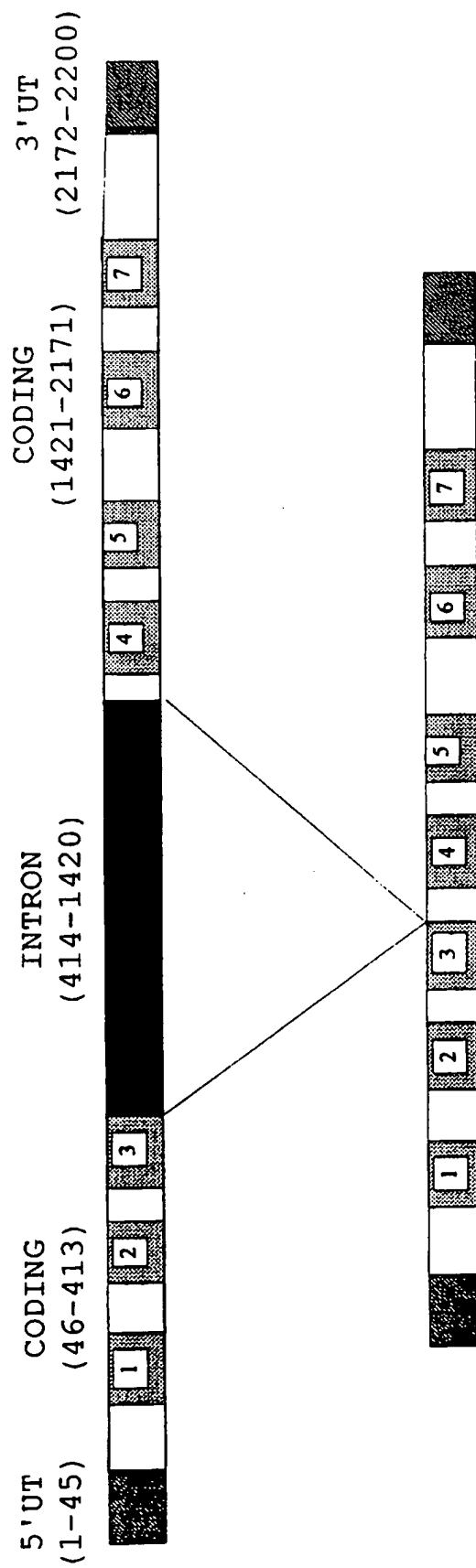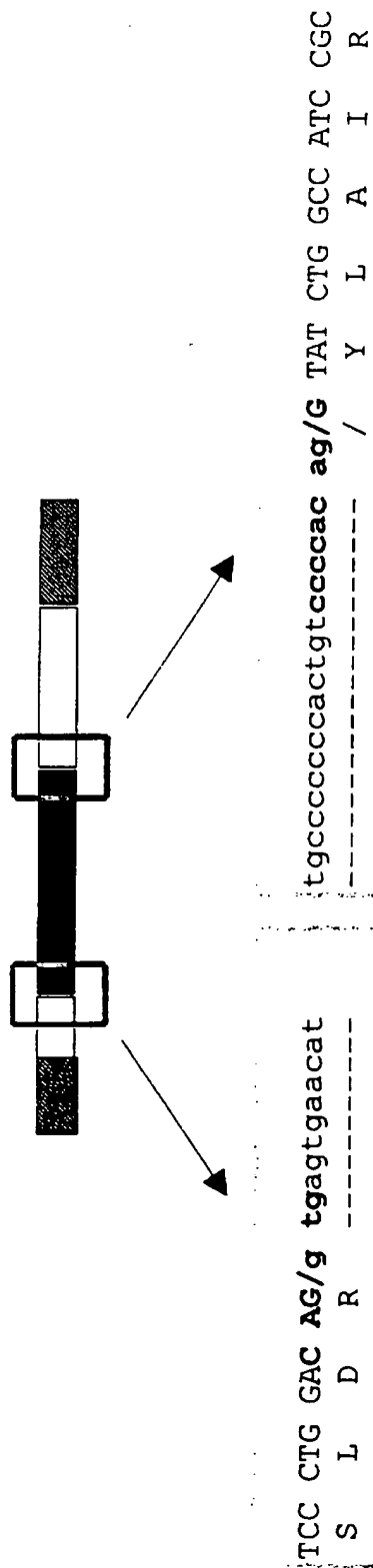
Figure 3A

Figure 3B

FIGURE 3C

```
414   GTGAGTGAACATCGGAGAACTATTGTATCTGAGATAGGGGCTTGGGCTGGAGTCACTACA   473
474   CAGGGGATCCAGAAGGCATGAGCAGCAGAAATGGGCGAGAACACTGAAATTACAAAGTGGCCTG   533
534   AGGCCGTGAAACGCAAGGGGGAGGGAGATTAAGACTCAGTGACTGAGAGTGTCTAAGTCG   593
594   ATGGGAGAAATCGGGTCCTGGGGTCCTCGCATTATTACTGCTTGAGTTAAATGTCTCTG   653
654   TGAAACATTGCAGTTCTCAGGCCAGAGTTGGCAGGAAAAGTAACTCGCCAGTGTTCAGAT   713
714   GCTGTTTGAGAGCTGCAGAGAAGCATCTGCTTCTTAGCACCAAGCTCAGCACCTGGGGCG   763
774   TTGTCCGGCGCCTTAGGACTGGGCTTGCTGTGTGTTAAGACCCATGCTCAAGTCC   833
834   AACGGAGTGTAAGCGAGGGCTCCTAGCTGACACCCAGAGCCCTCCAGGCCAAGGCTCCCC   893
894   TCACCGAGATGCCAGCGGTTTTATGCTCCTTCCATAGGTAAAGGACCCAGAAAGAAACAT   953
954   CCAGTATGCCCGGAGGGATCTTGACTGGGAAAAGACTGAATCCTGGTCTGGTGACCTTAGT   1013
1014  TCCCTGCCCTTTCACATCACTTGGACATTCCCACAGAAGAGCGGTGAAGAGGCGGTGGTC   1073
1074  CTTATTCTCCTCTGGTTTCCACTGAGTGCAACATGTGCGTCCTGAGTACGCTGGAGGGAC   1133
1134  TCACAAAATTTCAGCTTTCTTTAGGAGTTCCTTGCTGTAGTTTGACCCAAGTCTTCTCC   1193
1194  AGGTTTCTGTCAGAACCTGCAGGCATGAGGGGATCTGCCCTCCCCGGTTGTCACCAGAGGAT   1253
1254  AACAATCACTGCCCCAGAAATCCAGACAGATTCTACAACTTTTAGTCTTCGGTGTTTTG   1313
1314  GGGGTGCCCCTTCACGTGGAGTAGGTCGGTGGCCACATTCCCAGGAGTGACAATAGCCTA   1373
1374  GCAGTGAATCCCTCTCGCTTAGCTGATGCCCCCCCCACTGTCCCCACAG   1420
```
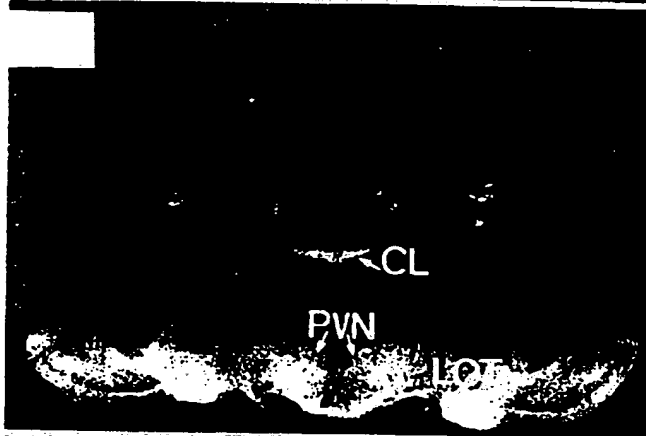
FIG. 4A-1

FIG. 4A-2

FIG. 4A-3
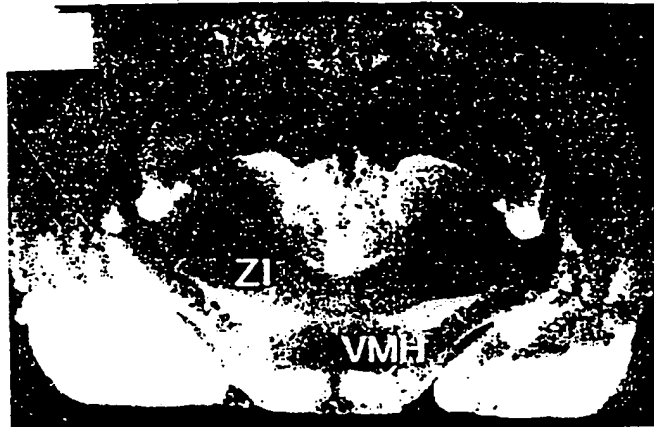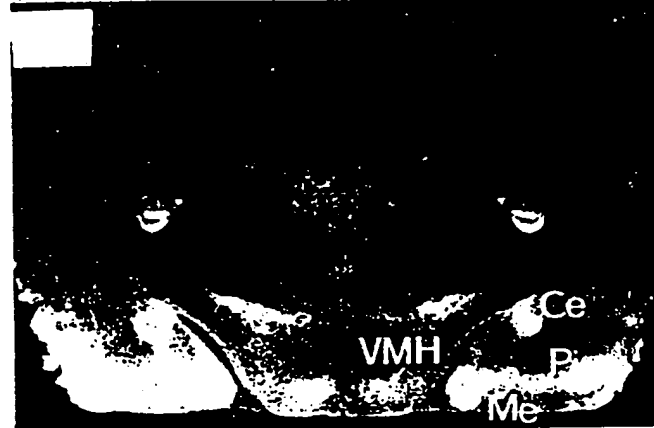
FIG. 4A-4

FIG. 4A-5

FIG. 4A-6
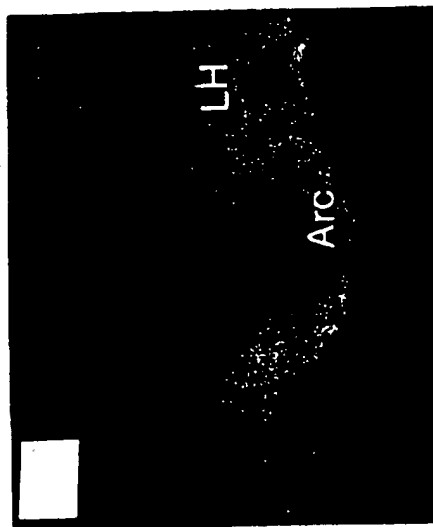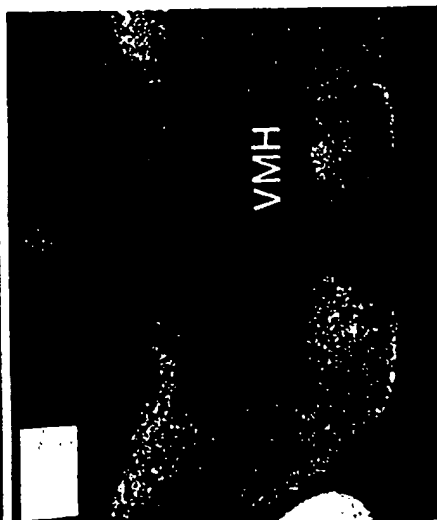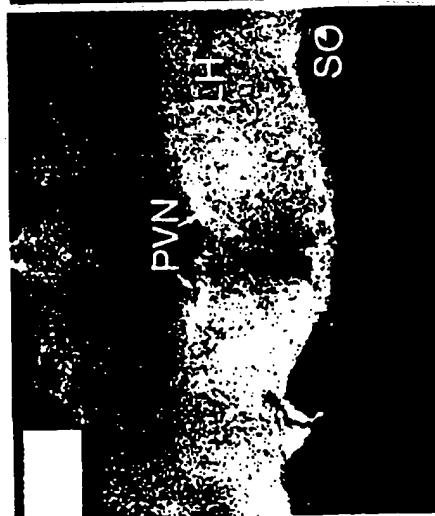
FIG. 4B-2
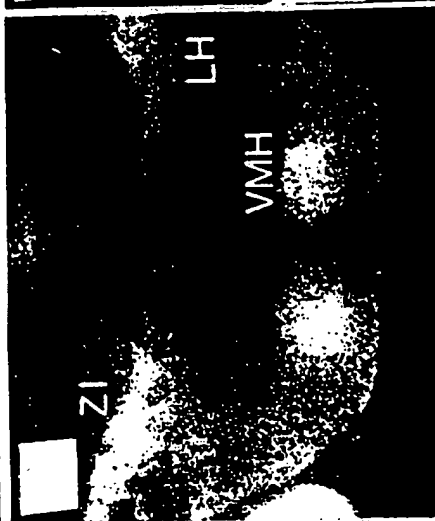
FIG. 4B-1
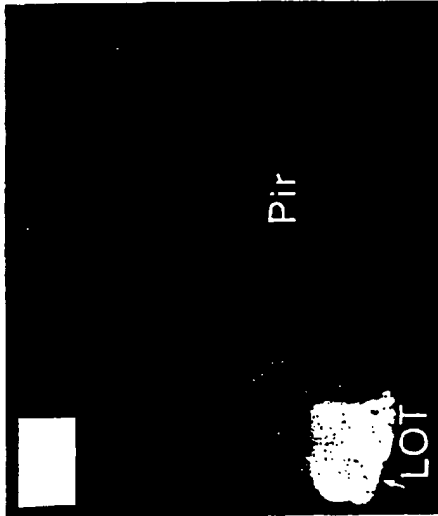
FIG. 4B-4
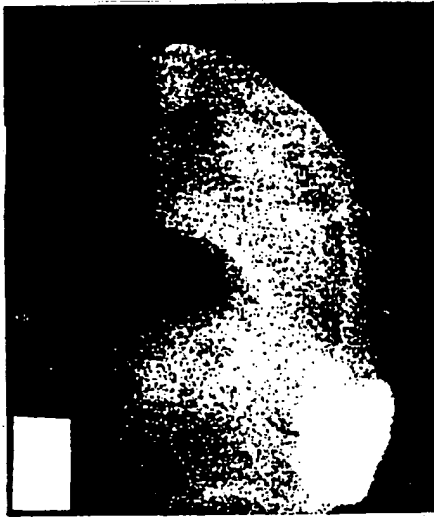
FIG. 4B-3
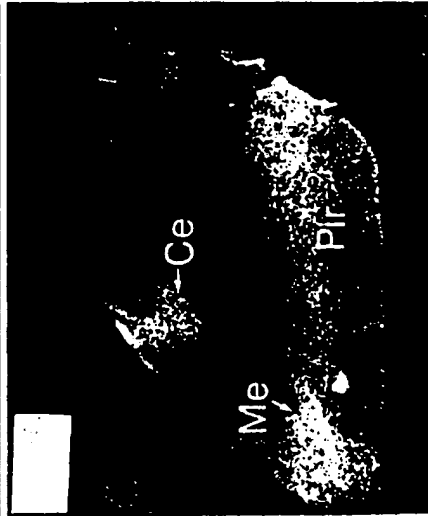
FIG. 4B-6

FIG. 4B-8

FIG. 4B-5
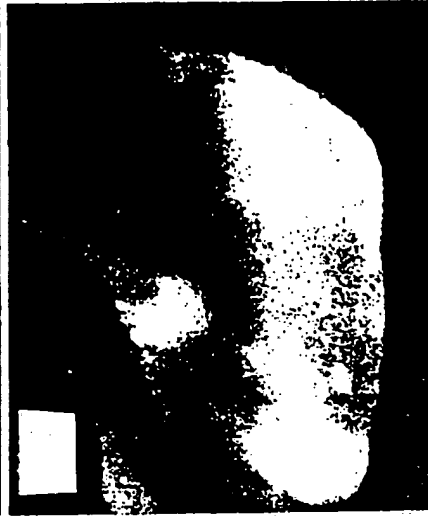
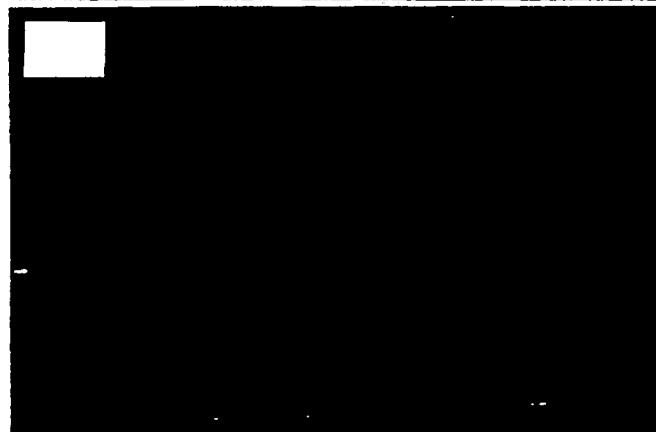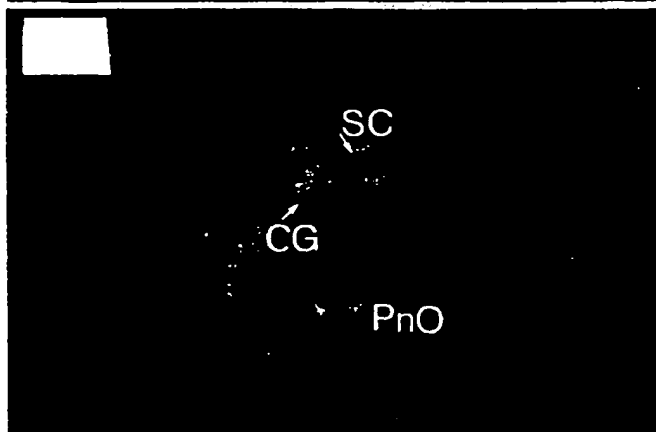FIG. 4B-7

FIG. 4C-1

FIG. 4C-2

FIG. 4C-3

FIG. 4C-4

FIG. 4C-5

FIG. 4C-6
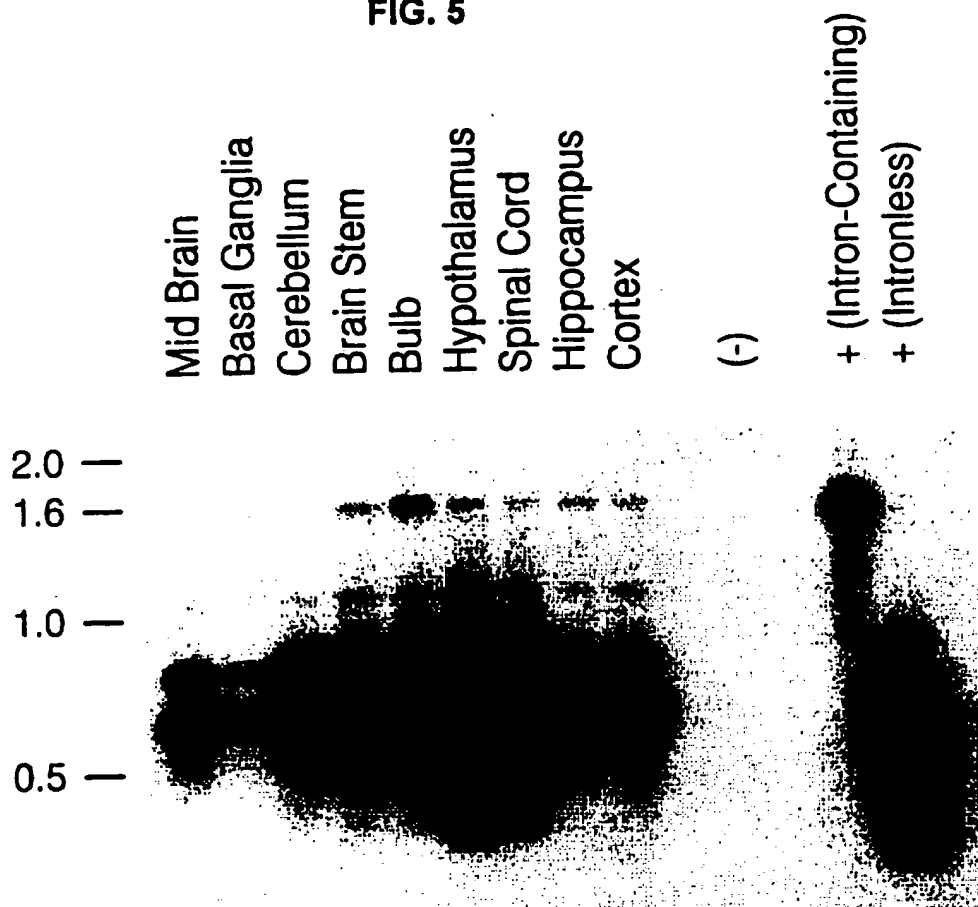
FIG. 5

FIG. 6A

FIG. 6B

FIG. 7A

Heart
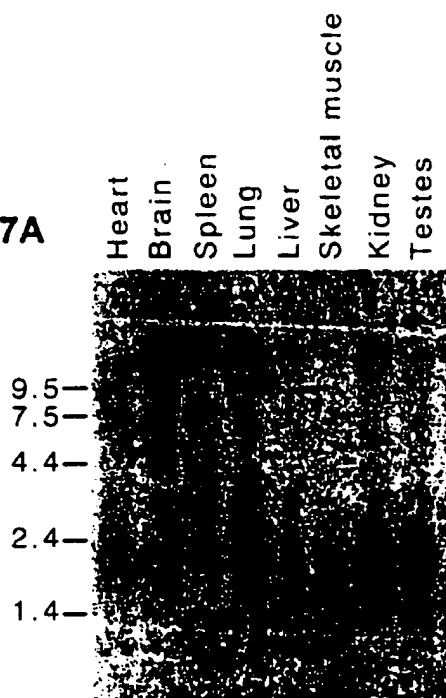Brain
Spleen
Lung
Liver
Skeletal muscle
Kidney
Testes

9.5—
7.5—
4.4—
2.4—
1.4—

FIG. 7B

FIG. 8A

FIG. 8B

FIG. 8C

FIG. 8D

FIG. 9A

EC$_{50}$ = 0.26 nM

cAMP Formation
(% Forskolin Response)

Log[pGalanin$_{(1-29)}$] (M)

FIG. 9B

EC$_{50}$ = 112 nM

[^{3}H]InsPO$_4$
(cpm/well)

Log[pGalanin$_{(1-29)}$] (M)
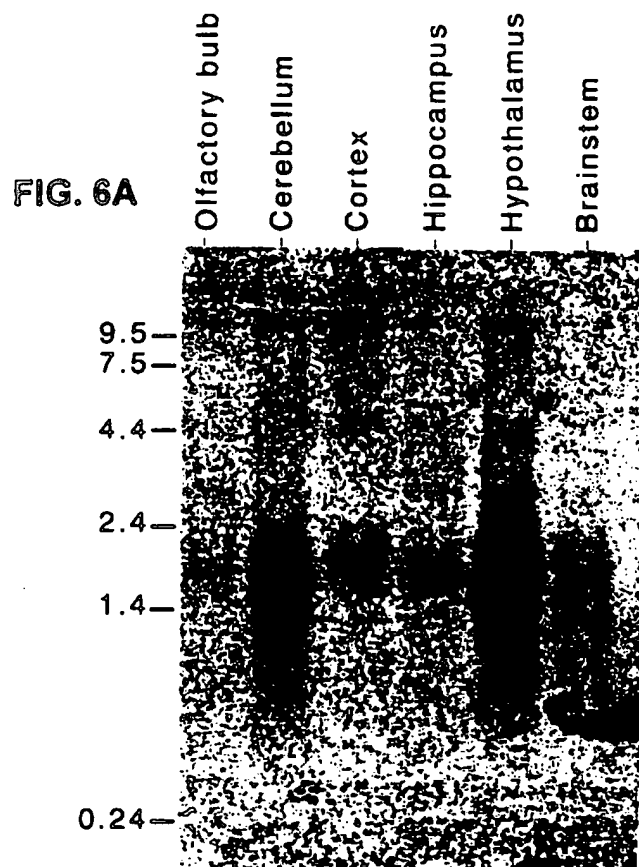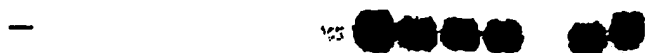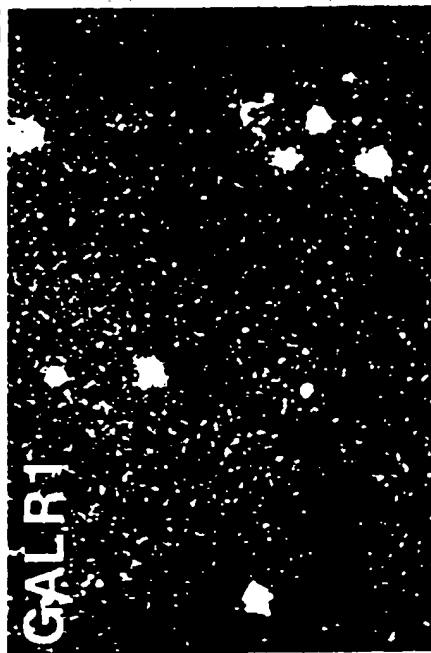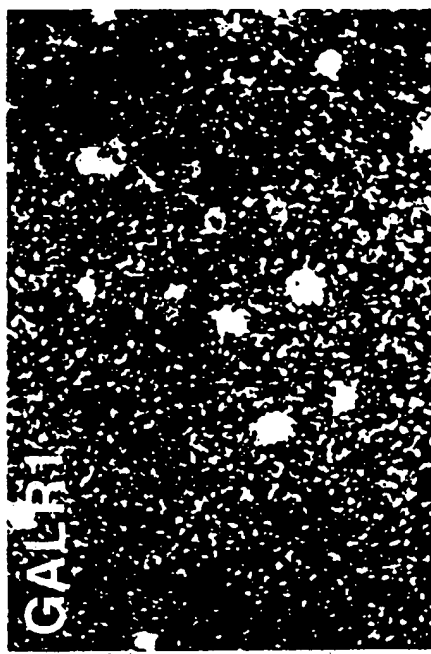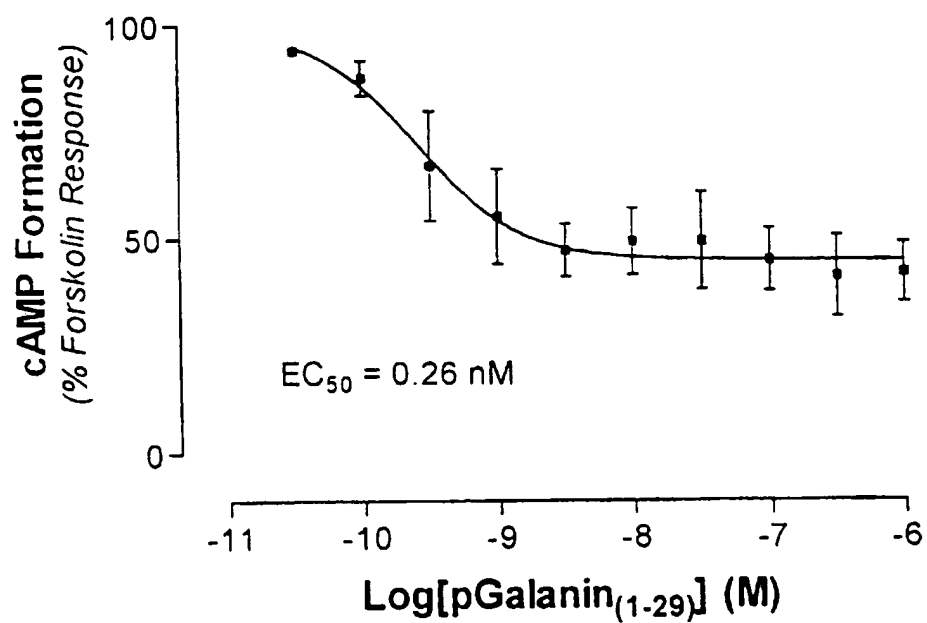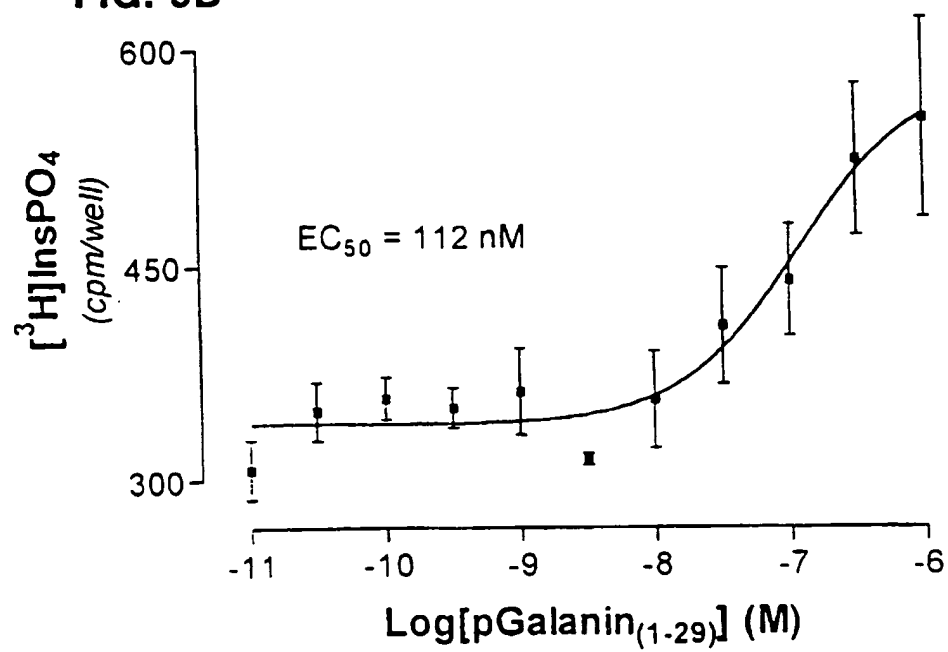
FIGURE 10

```
-100   AGTCGCACTAGGAGTTGCAGCGGCCCGGGAGCTTCCCGCTCGCGGAGACCCAG          -41
 -40   ACGGCTGCAGGAGCCCGGGCAGCCTCGGGGTCAGCGGCCACCATGAACGTCTCCGGCTGCC   19
  20   CAGGGCCGGGAACGCGAGCGCAGGCCAGGCGCGGGGGAGGCTGGCACCCCGAGGCGGGTCA   79
  80   TCGTGCCCCTGCTCTTCGCGCTCATCTCCGTGGGCACCGTGGGCAACACGCTGGTGC      139
 140   TGGCGGTGCTGCGGGCGGTGCTGCCAGGCGGTCAGCACTACCAACCTGTTCATCCTTAACC  199
 200   TGGGCGTGGCCGACCTGTGTTCATCCTGCTGCTGCCTGCTGTGCAGGCCACCATCTACA    259
 260   CCCTGGACGCTGGGGGTGGGTGTTCGGGCTCGCGTGCCGCCGTCTCCCTGGACAGGTATCTTCC 319
 320   TCACCATGCACGCCAGCAGCTTCACGCTGCACTCCCGAGCTGGCCGTCTCCCTGAAACGCG   379
 380   TCCGCTACCCGCTGTCGCGGGGCTGCTGCTTCTTCCGGGCCCTACCTGAGCTACCGCGCCAGT 439
 440   GGCTCATCTGGGGGCTGCCAACCTTGCGCTACTACCTTGCGCTCTTCAGCTACCGTG      499
 500   CGCAGCTGCCAACCTGCCAAGCGCCAGTGGGCCCCCTCGCCCGCGCGCCCA            559
 560   TGGACATCTGCACCTTCGTCTGCGCTGCTTCCTGTTCTCGGCGGCTCGGGGTG          619
 620   ACGCGCGCCACCTTGCGCTACTACGCGTGGCCGTCAGCACACGATCCTCATCGTGGCGACCATCT 679
 680   CCCGGCGCGCCAAGCGCAAGTGACACGCGCTCATCCTCTGCGGTGGTTCGCGCCAGTTCCCGCTCA 739
 740   TCTGCTGGATGCCCCACCACGGCGCTCATCCTCGGTTCGCGCCACCTGGTGGTTCGGCCA      799
 800   CGCGCGCCACTTATGCGCTTCGCATCCTCTCGCTGGTCTCTCGCACCTGGTCTCTCGCACGATCCTGCG 859
 860   TCAACCCCATCGTGTTACGCCTGGGCGCTGGGCCCCCAGGCCGTGCCGAGCGCTTCCGCACGATCT 919
 920   GCGCGGCACCCAGCTGCCTGGGCGTGGCCAGCGGCCGTGTGTGCCGCGCG              979
 980   GGGGCACCCACAGTGGCGGGGCCCTTCGTCGCTGCCCCGGCCCGAGTCCTGCCCGGCTGCCATGAGCG 1039
1040   AGGCGGGCCCTTGGAGGCGCCCAGGCGCCCAAAGGCAGGCAGCGACCGTTGCACATGAGCCCT 1099
1100   GTCCTGCCCGTCCTGGCAGGGCGCGCTGGGGATGTCACAGGCCATCCTGACGGTTGATGTGG 1159
1160   CCTGAAAGCACTTAGCGGCGCGCTGGGAAGGCTGGAGTCACAGAGTTGGAGTCATTGTTGGGGAC 1219
1220   CGTGGGGGAGAGCTTTGCCTGTTAATAAAACGCACAAACCATTTCA                 1264
```

FIGURE 11

```
  1   M N V S G C P G A G N A S Q A G G G G G    20
 21   W H P E A V I V P L L F A L I F L V G T    40
 41   V G N T L V L A V L L R G G Q A V S T T    60
 61   N L F H L N L G V A D L C F H L C C V P    80
 81   F Q A T H Y T L D G W V F G S L L A K A   100
101   V H F L L F L T M H A S S F T L A T V S   120
121   L D R Y A A I R Y P L H S R E L R G P R   140
141   N A L A R I G L H W G L S L L F S A P Y   160
161   L S Y Y R Q S Q L A N L T V C H P L W S   180
181   A P R R L A M D H C T F V F S Y L V P V   200
201   L V L G S T Y A R T L R Y L W R A I D P   220
221   V A A G F G A R R A K R K V T R M C L I   240
241   V A A L P C L C W M P H H A L I L H V W   260
261   F G Q F S L T R A T Y A L R H L S H L V   280
281   S Y A N T C V N C I V Y A L V S K A P R   300
301   K G F R A I C A H L L G R A P G R E S G   320
321   R V C A M A R G T H S G S V L E P A S S   340
341   D L L H E S E A A G A L R P C P G G S Q   360
361   P C H L D P C P G P S W Q G P K A G S Q   380
381   I L T V D G S                            387
```

Figure 12A

Figure 12B

TCC CTG GAC AG/g tgagccagcgc - - - - - - - - - - tcccttcccggtctgaccgc ag/G TAT CTG GCC ATC CGC
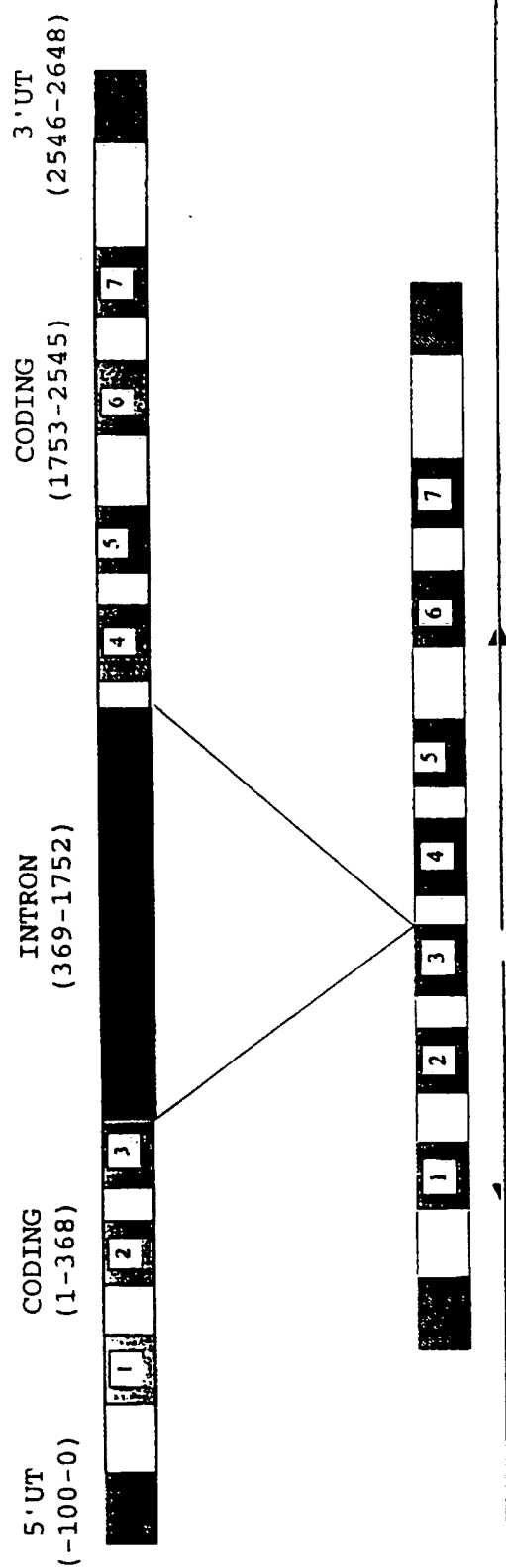 S   L   D   R   /   Y   L   A   I   R
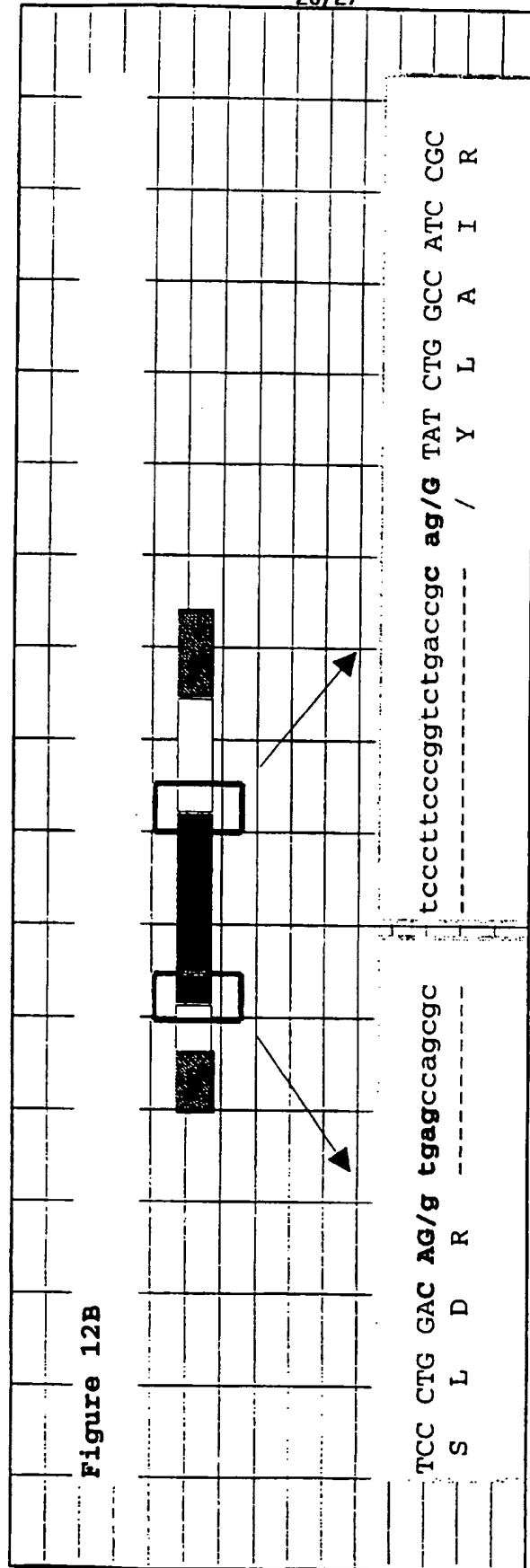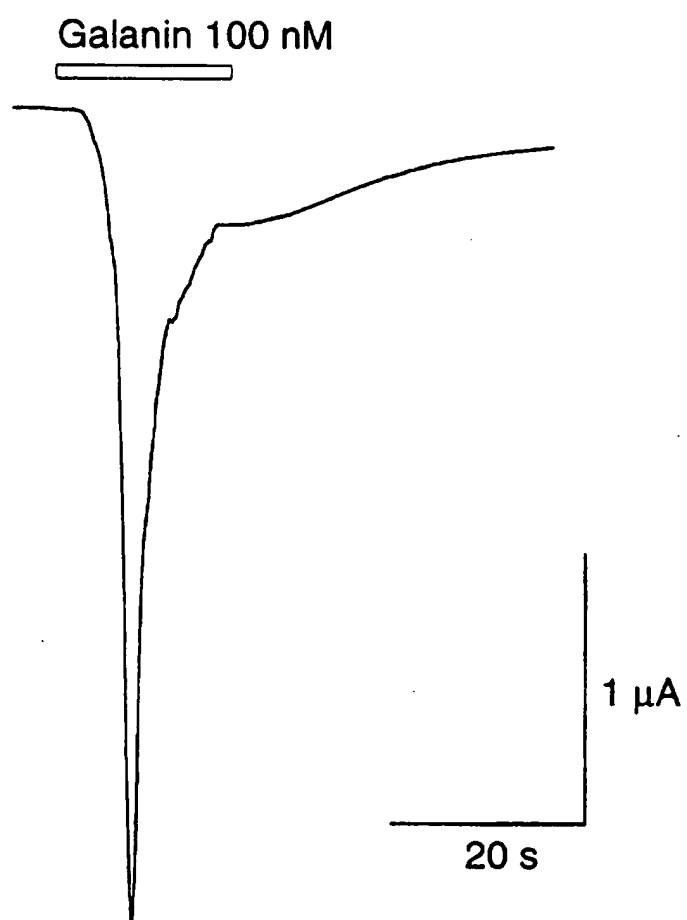
FIGURE 12C

```
 369  GTGAGCCAGCGCCTTGGCCTCCCTGGGAGATGGCATCCACGCGGGGGATGGAGCGGGAG  428
 429  GCGGGACTGGGGGACCAAGAAGGACGCGCAGAGTGGGACACTAGGCAGAGGCAGTGG     488
 489  AAGACAAGCGGGCGCGGAGGAGGAAAAAGAGGAATAAGAGGAATTGGGGACCGTGTCCCT  548
 549  CGGTTAGATGCGTCCTGGGGCCTGGAAGCCTGGAGAATGTGCTCTCCAGCGCCGCCCGT   608
 609  GCCTGACAACGCGCAGCGTTCCCAGTACGACGCGGTTGTGCGCGTTCATCTCGCTTGAG   668
 669  CTTAATGCCCTCCGTGAGGGTGGGATAGGACACAAAGTGCCCAATATACAGAAGAGTTGAGT  728
 729  TCCTAAGTAACTCGCTCAGAGTCGCCAGCAGGGATCGCGCCTCGGGTGCGTGAAGTGACCGTCTGT  788
 789  CTCCTGCGAGCCAACTTCAGGCGCGCTCCACTGCGTCCTTGGGTCCTTGCAAGGATCCACTCC      848
 849  TTGGTGCAGCTGGCTCAGGTCCAGGCTGTGATCTGTGGGTCCCTAGCTCAGTCCCACTCTGCCTCTCG  908
 909  GGAGTCCCAGCGAGCCGTGCCTAAAGGTGCCTAGCTCAGTCCCTAGCTCAGTCCCACTCTGCCTCTCG  968
 969  CCTCCAAAACAAACAAAACAAAATAAAATCCAAAACAAGTCGGGCCGGGAGAGAGCGT    1028
1029  GCCCTGGGGTTCTTCCTCCCCAGCAGAGGAGCGCACATTCGGGAGAGCGC          1088
1089  GGGACTCAGGTGGAGCTTGAAAGGACACTGGGATGGTTCCTGGGGAAATCCGGGTAT      1148
1149  TTCCCCCTCCATCCTCTGGAAAAAACAGAGAGGCCAGACTGCCCCCACACCTCCT      1208
1209  GTAGCCACTGAGCGCGAAGTGCGTTGGTTCCGAGCGCGCTGGTGGGATCCAACAAAGCTCG   1268
1269  CATTCTTCTCAGGAATCCCCTGAGAAATTAACTGTCCCTGCCCAACATGTCTTCTCCAGG    1328
1329  CTGTCTGCTAGAGCCTCAGGCGCTCCCATAGCCGGTTCTCCCACCGGTCGTCACCAGTGGGT  1388
1389  AGTCACAGCCTCCCCGGAGCCCCATAGCCGGTTCTCCCAACCTTTAGTCTTCAGTGGCTTTGG  1448
1449  GGTGCCCCTCTCAGTGAGACTGTGGTTGCAGTCCCCGGGGCAGCGGGAGAATGGCTTGA     1508
1509  AGGCACACCTTCCTGCGCCGGCCCCCCATTCCAGGCTCCGCTGAGTGTCTGGGA        1568
1569  CACGCTGGAGGCCCCCCACCCTCCGCCGAGCCTCACCCCACCTCCTCGTGT          1628
1629  GCGGTGTAACCATGCGCTAAGGACCTTCCTCGGAGAGCCGGAGGTGCAGGG        1688
1689  GTCGCGGCCCTCAGCCGACGTCTCCCGGTCTCCCGGTCTGACC              1748
1749  GCAG                                                    1752
```

FIGURE 13

Galanin 100 nM

1 µA

20 s
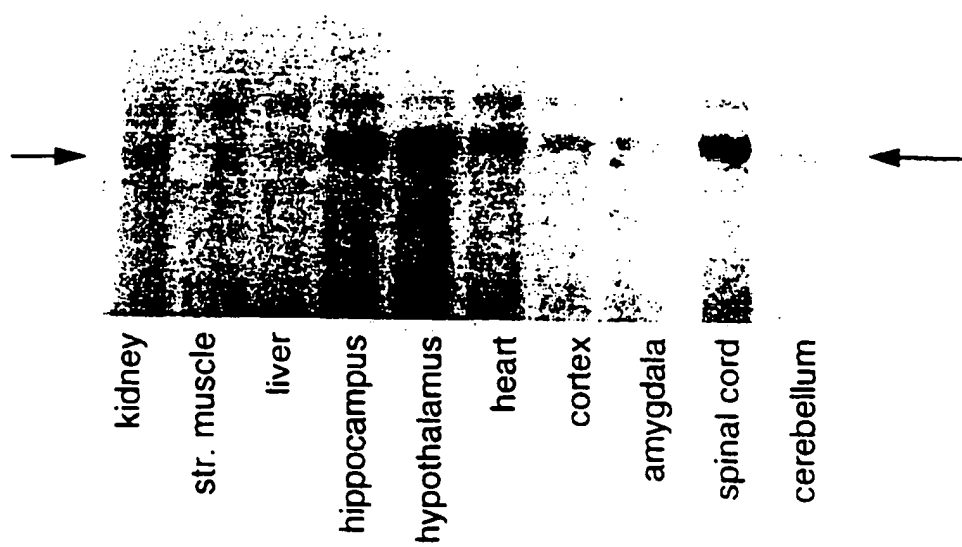
FIGURE 14

Figure 15A

```
rGALR2  . . . M N G S G S Q Q A E N T S Q E G G S G .      19
hGALR2  . . . M N V S G C P G A G N A S Q A G G G G .      19
rGALR1  M E L A P V N L S E G N G S D P E P P A E P R P L  25

                                       I
rGALR2  . G W Q P E A V L V P L F F A L I F L V G T V G N  43
hGALR2  . G W H P E A V I V P L L F A L I F L V G T V G N  43
rGALR1  F G I G V E N F I T L V V F G L I F A M G V L G N  50

rGALR2  A L V L A V L L R G . . G Q A V S T T N L F I L N  66
hGALR2  T L V L A V L L R G . . G Q A V S T T N L F I L N  66
rGALR1  S L V I T V L A R S K P G K P R S T T N L F I L N  75
        II
rGALR2  L G V A D L C F I L C C V P F Q A T I Y T L D D W  91
hGALR2  L G V A D L C F I L C C V P F Q A T I Y T L D G W  91
rGALR1  L S I A D L A Y L L F C I P F Q A T V Y A L P T W  100
```

Figure 15B

```
                              III ─────────────────────────
rGALR2  V F G S L L C K A V H F L I F L T M H A S S F T L   116
hGALR2  V F G S L L C K A V H F L I F L T M H A S S F T L   116
rGALR1  V L G A F I C K F I H Y F F T V S M L V S I F T L   125

rGALR2  A A V S L D R Y L A I R Y P L H S R E L R T P R N   141
hGALR2  A A V S L D R Y L A I R Y P L H S R E L R T P R N   141
rGALR1  A A M S V D R Y V A I V H S R R S S L R V S R N     150

        IV ──────────────────────────────
rGALR2  A L A A I G L I W G L A L L F S G P Y L S Y Y R Q   166
hGALR2  A L A A I G L I W G L S L L F S G P Y L S Y Y R Q   166
rGALR1  A L L G V G F I W A L S I A M A S P V A Y Y Q R L   175

rGALR2  S Q L . A N L T V C H P A W S A P . R R R A M D L   189
hGALR2  S Q L . A N L T V C H P A W S A P . R R R A M D I   189
rGALR1  F H R D S N Q T F C W E H W P N Q L H K K A Y V V   200
```

Figure 15C

```
        V
rGALR2  C T F V F S Y L L P V L V L S L T Y A R T L R Y L  214
hGALR2  C T F V F S Y L L P V L V L G L T Y A R T L R Y L  214
rGALR1  C T F V F G Y L L P L L I C F C Y A K V L N H L    225

rGALR2  W R T V D P V T A G S G S Q R A K R K V T R M I I  239
hGALR2  W R A V D P V A A G S G A R R A K R K V T R M I L  239
rGALR1  H K K L K N M S K K S E A . S K K T A Q T V L      248
              VI
rGALR2  I V A V L F C L C W M P H H A L I L C V W F G R F  264
hGALR2  I V A A L F C L C W M P H H A L I L C V W F G Q F  264
rGALR1  V V V V F G I S W L P H H V I H L W A E F G A F    273
                                   VII
rGALR2  P L T R A T Y A L R I L S H L V S Y A N S C V N P  289
hGALR2  P L T R A T Y A L R I L S H L V S Y A N S C V N P  289
rGALR1  P L T P A S F F F R I T A H C L A Y S N S S V N P  298
```

Figure 15D

```
rGALR2   I V Y A L V S K H F R K G F R K I C A G L L R P A   314
hGALR2   I V Y A L V S K H F R K G F R T I C A G L L G R A   314
rGALR1   I I Y A F L S E N F R K A Y K Q V F K C R V C N E   223

rGALR2   P R R A S G R V S I L A P G N H S G S M L E Q E S   339
hGALR2   P G R A S G R V C A A A R G T H S G S V L E R E S   339
rGALR1   S P H G D A K . . . E K N R I D T P P S             340

rGALR2   T D L T Q V S E A A A G P L V P P P A . . . L       358
hGALR2   S D L L H M S E A A A G A L R P C P G A S Q P C I L 364
rGALR1   T N C T H V                                         346

rGALR2   P N C T A S S R . . . . . T L D P A C               372
hGALR2   E P C P G P S W Q G P K A G D S I L T V D V A       387
```

Laurence H. Posorske   I.D. No. 06592

Whole Cell Immobilization
Chloroplast Techniques
Spin Label
Spin label Paper
High Stress Rx^4 Conditions
SWECO
Delaval Evaporators & Dryers
Techniques Chromatography
NMR-PRR, Etc.: COHN
NMR

Laurence H. Posorske   I.D. No. 06592

LIPID Analysis - HPLC
Techniques Carbohydrates
Pectinase Assays
Lipid Anal. Proc
Lipide Anal GLC
Biochem Handouts
Techniques - Centrifugation
Super Critical Extraction
Techniques - Viscometry
Continuing Education
Patent Misuse
Exp Design Notes
Exp Design Course
Business
Techniques - Protein Modification
Kinetics & Binding